THE AUTONOMIC INNERVATION OF SMOOTH MUSCLE

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THE AUTONOMIC INNERVATION OF SMOOTH MUSCLE

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PREFACE

"Within recent years our knowledge of the autonomic nervous system has been greatly increased, but there is still much to be discovered. There is also a great deal to be forgotten--inaccurate anatomical observations, physiological experiments based on false anatomical conceptions, and unsound surgical procedures founded on inadequate knowledge of both anatomy and physiology. The seeker after truth in the literature on the autonomic nervous system has no easy task, and he is apt to be confused by conflicting statements. The difficulties of those who tackle the problem directly are equally great, for the complexity of the intercommunications within the system and the small size of many of the nerves combine to render direct anatomical study difficult, and lack of accurate anatomical knowledge renders physiological investigations of less value than they otherwise would be."--Mitchell, G. A. G. (1935b).

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INTRODUCTION

Literally thousands of articles have been written on the autonomic nervous system, describing the gross anatomy, histological structure, physiological nature, connection with the central nervous system, etc. Many of the previously accepted conclusions have been disputed in recent years. The gross anatomy of the autonomic nervous system and the connections with the brain and spinal cord have been described in detail by such authors as Langley and Kuntz; however, little progress has been made in the description of the endings of these nerves on smooth muscle. Consequently, it is my purpose in this paper to discuss the innervation of smooth muscle from the physiological and particularly the histological aspects.

The autonomic or involuntary nervous system governs those functions of the body which are carried out automatically or reflexively, and ordinarily do not involve conscious intent. Among its functions are the regulation of the movements of the heart and gastrointestinal tract, the control of the smooth muscle of the blood vessels, urinary bladder, bronchi and skin, and the activities of the various glands. The connection of the autonomic system with the central nervous system and its anatomical divisions are well-known, and Kuntz (1934) has described the gross anatomy of the system. The autonomic pathway consists of at least two neurons, the preganglionic, with cell body in the central nervous system, and the postganglionic, with cell body in either a lateral ganglion, a collateral ganglion, or in the wall of the effector organ.

The grouping of the autonomic nervous system into two antagonistic systems, the sympathetic and parasympathetic, is not entirely anatomical, but is in part physiological. The theory of synaptic transmission postulates

that a chemical, acetylcholine, is responsible for the transmission at ganglionic synapses. In most cases, this substance occurs at the junction between parasympathetic fiber and effector. Consequently, the parasympathetic system is cholinergic. However, as Cannon and Rosenblueth (1937) and others have shown, sympathin, similar to adrenalin, is produced at the endings of the postganglionic fibers of the sympathetic system. Thus the sympathetic system is adrenergic. It will be seen later that the two classifications, physiological and anatomical, are not synonymous.

The plexuses of Auerbach and Meissner are often described as belonging to the autonomic system. These are networks of nerve fibers, with ganglion cells, in the walls of the digestive organs, and are included provisionally in the autonomic nervous system under the term enteric autonomics. Langley (1922) describes this enteric plexus, and in particular its connections with the finer branches of the vagus supply to the gastrointestinal tract. Best and Taylor (1938) state that the short postganglionic fibers of the vagus form a rich plexus, Auerbach's plexus, in the walls of the gastrointestinal tract, bronchioles and biliary ducts. This will be discussed in more detail later.

Cannon and Rosenblueth (1937) summarize the general functions of the autonomic nervous system. They say that this system provides for those adjustments which, in the most highly evolved animals, "maintain the stable states of their internal environment." Generally, its prime function is "the maintenance of stability or homeostasis in the fluid matrix of the organism," although there are some functions which lie outside this broad generalization, for instance, the emptying of the pelvic organs and the protective action of the nictitating membrane and the lachrymal glands. The

action of the sympathetic system allows a widespread increase or decrease of function in its effectors; on the other hand, the parasympathetic system deals with its effectors not in a great group, but separately.

It has been shown that the sympathetic division is not essential for existence, but mainly for maintaining homeostasis. The cranial division may be considered as "protective, conservative and upbuilding" in its services to the organism; the sacral division of the parasympathetic system serves mainly for the reflex emptying of the hollow pelvic viscera.

In accordance with Sheehan's (1941) suggestion, only the efferent neurons shall be described as truly autonomic. While the sensory afferent neurons course with the efferent fibers, they are probably related to somatic afferent neurons, and are therefore not to be considered as autonomic.

SMOOTH MUSCLE

Before describing the autonomic innervation, it is desirable to review briefly the properties and location of smooth muscle. The smooth muscular tissue is so called because its stained preparations do not exhibit the cross striations characteristic of voluntary and heart muscle. The individual cells are spindle-shaped, from .015 to .5 mm. long and .002 to .02 mm. in diameter, and possess a single large nucleus. The actual intracellular structure, especially with regard to the contractile mechanism, is disputed. Many histologists have shown the presence of fine longitudinal fibrils in fixed tissue. These fibrils have been suggested as the contractile units. However, since these fibrils have been demonstrated only in fixed tissue, they may be artifacts resulting from the coagulation produced by the fixing agent. McGill(1909), in discussing the structure of smooth muscle of various chordates in the resting and in the contracted condition, describes two types of myofibrillae, namely a very fine type, and a large, coarse one. Either or both may be present in one cell. When fibers are found only at the periphery of the cell, they are invariably of the larger type. They do not vary in caliber within the cell, and they run from one cell to the next through the anastomoses which she has described in the majority of the smooth musculature. She shows two types of contraction, both involving the myofibrillae and the remainder of the cell, namely, a total contraction, in which the entire fiber shortens and thickens, and a peristaltic contraction, in which a series of wave-like thickenings cross the fiber, giving it the appearance, with proper technique, of striated muscle. In an earlier paper, McGill (1907) described the formation of the smooth muscle in the intestine of the pig from a mesenchymal syncytium.

During the process, the mesenchyme seems to condense as its nuclei elongate. While this is taking place, the myofibrillae begin to appear in the cytoplasm surrounding the nucleus. They arise as a series of "coarse varicose deeply-staining fibrillae". In later development they may or may not break down to form finer fibrillae.

Tiegs (1924) has described the fibrillae in the smooth muscle cells as the contractile elements, and has cited two excellent observations in support of his statement. In the first place, he has observed that in hypertrophied cells (for example, those of the pregnant uterus) the fibrils are larger than in the resting cells. This fact is hard to explain in considering these elements as coagulation artifacts. In the second place, he has compared muscular cells from regions of contraction with those from regions of relaxation, and has found that the myofibrillae in the former are considerably thicker. Many authors object to the theory of contraction of myofibrillae on the grounds that myofibrils are not visible in the living cell. However, Tiegs claimed the refractive index of the myofibrils is so nearly that of the remainder of the cell that they are invisible.

Since the nucleus of the smooth muscle fiber occupies a central position, it follows that the myofibrillae are peripheral in location, for they do not approach the nucleus. The nucleus is surrounded by an area of undifferentiated cytoplasm, in which changes resulting in contraction or relaxation first take place.

The nature of the smooth muscle cells in tissue has long been a point of debate. Engelmann (1870) is probably the first to have postulated a syncytial connection between smooth muscle fibers. Since his time, two schools of thought have arisen. The one, headed by Evans (1926) and Bozler

(1938, 1939), theorizes that the smooth muscle, particularly of the uterus, is syncytial in nature. The other believes that smooth muscle is not syncytial, but is composed of independent units.

In reviewing the evidence presented by the first school, the history of the theory of intercellular bridges in the smooth musculature will be outlined. Barfurth (1891), de Bruyne (1892), and Werner (1894) have all reported the presence of fine protoplasmic bridges in many cases. McGill (1907, 1909) follows the development of the smooth muscle cells of the alimentary canal from a mesenchymous syncytium which surrounds the embryonic entoderm. She suggests that remnants of the syncytium persist as the intercellular bridges of adult tissue, and describes two, and possibly three, types of structure of the smooth muscle. Type 1 is distinctly syncytial, with end and side anastomoses. This type is found in portions of the digestive and urogenital tracts of adult Necturus and various mammals. Type 2 consists of numerous end to end anastomoses while the side anastomoses have decreased in number. Type 3, commonly described, but demonstrated by her with difficulty, consists of isolated muscle cells. She reports that the myofibrillae and probably also the granular cytoplasm are continuous from cell to cell through the anastomoses. Evans (1926) seems to agree that some smooth muscle may be syncytial, but goes on to suggest that there may be more than one kind of smooth muscle. This is indeed true as far as embryonic development is concerned, for in some instances (muscle of sweat glands, sphincter and dilator pupillae muscles) the muscle is derived from ectoderm although commonly it arises in the mesoderm layer.

Jordan (1937), in describing the histology of smooth muscle, suggests that there may be two kinds of smooth muscle fibers; the visceral kind,

which is found in the uterus and ureter, and is probably syncytial, and the type with motor innervation, which he says is probably not syncytial. He draws physiological and histological analogies between cardiac and visceral muscle.

Bozler (1938, 1939) offers experimental evidence which shows that the uterine musculature, unlike that of the majority of the visceral organs, is syncytial. He shows that a weak electrical stimulus applied to an excitable uterine strip is propagated over the entire preparation. By varying the stimulus, both in intensity and in location, he demonstrates that there is no nervous mechanism involved, and that the stimulus must pass directly from cell to cell. He later extended these studies to the musculature of the gastrointestinal tract, and concludes that this muscle is also syncytial.

Morison (1940), from his investigations on the uterine musculature during oestrus, concluded that the uterus may behave as a single unit, with the constrictions conducted throughout the muscle.

Fulton and Lutz (1942) report the vascular responses in preparations of the frog's retrolingual membrane in which the nerve plexus had been made non-functional by cocaine. Since the response is found in exactly the same area which had responded to nerve stimulation before treatment, and since the possibility of nerve conduction had been eliminated, this limited conducted response implies a non-nervous conducting mechanism, such as a muscle syncytium, discontinuous at the junctions of certain vessels. Therefore the smooth muscle motor unit may consist of many cells connected through a syncytium similar to that already reported in the uterus.

On the other hand, there is the school of thought which believes that smooth muscle is not syncytial, but is composed of independent units. In 1924,

Tiegs published the first of a series of papers on the smooth musculature. He found that there was no transmission of stimuli within the muscle fibers or from fiber to fiber, and that the innervation of these cells was rich enough to allow for transmission.

Eccles and Magladery (1937b) describe smooth muscle units, in the nictitating membrane which probably consist of one cell. They find that in contraction these units set up beats in adjacent units. Thus they do not believe in a syncytium, but in a 'humoral' transfer of impulse from cell to cell. Cannon and Rosenblueth (1937) state that they find no support for the presence of a syncytium like that of the heart in the smooth musculature, at least in that of the nictitating membrane.

It is apparent from this brief review of the work done on the nature of the smooth musculature that there are two types of smooth muscle, the one syncytial and the other consisting of independent cells. In the former case transmission of impulse from cell to cell is direct; in the latter case there is either a humoral transfer of impulses, or a nerve network, similar to the terminal reticulum described by Boeke (1940), which transmits the impulse from cell to cell.

Authors are also in disagreement as to the myogenic nature of the pericapillary cells. Since Rouget published his famous paper describing the cells which have become known by his name, the function of these cells during the activities of the capillaries has been investigated by many workers. Krogh, Vimtrup and others state that the Rouget cells are modified muscle cells, and that they are responsible for the contraction of the capillaries, in part if not entirely. Clark and Clark, Zweifach and others postulate that the pericapillary cells are not contractile, and that any

response of the capillaries to vasomotor stimulation cannot be attributed to these cells.

Clark and Clark (1925b), in observing the non-pigmented adventitial (Rouget) cells on the walls of the blood capillaries of the tadpole's tail, came to the conclusion that the capillary endothelium has a definite tonicity and contractility, independent of any Rouget cell. During capillary contraction, they report that the capillary itself apparently drew away from the Rouget cell, leaving a perceptible space between the vessel and the cell. In 1940 they added, from observations made on living tissue, that the longitudinally-arranged adventitial cells on capillaries and small venules are not contractile. They observed the development of the muscular cells on the small arteries and arterioles, and stated that these became contractile when they were reached by a regenerating vasomotor nerve.

Florey and Carleton (1926), reporting on the active contraction and dilation of capillaries, conclude that the Rouget cells are not present in sufficient quantities to provide for this action, and that the endothelial cells must therefore be responsible for the motor activity of the capillary.

Michels (1936), in his investigations on the capillaries of the omentum of the rabbit and of the web of living frogs, found no evidence for the muscular character of pericapillary cells. He suggests that, because of the variations in the description of 'Rouget cells', this term should be dropped from the literature, and the word 'pericyte' used in describing pericapillary connective tissue cells.

Zweifach (1934) reports that neither a muscular coat nor any muscle elements are found on the capillaries. Zweifach (1937) and Zweifach and Kossmann (1937) describe two types of capillaries, namely muscular capillaries

or arterio-venous bridges, and endothelial or true capillaries. The first will be discussed later; the true capillaries are stated to be devoid of contractile pericapillary cells.

Moon (1938) points out that the reaction of the Rouget cells to histamine is in direct contradiction to the theory of their derivation from smooth muscle. It is well known that histamine, even in low concentrations, will cause contraction of smooth muscle cells, including those of the arterioles; however, when capillaries are treated with histamine they dilate, so that the response of the contractile elements, whether Rouget cells or endothelium, is not that characteristic of smooth muscle.

Moon states that the disagreement as to the site of capillary contraction "does not concern the fact of independent capillary contractility, but concerns only the mechanism by which it is accomplished." Thus the authors agree that capillaries can contract independently of their supplying arterioles, but are unable to agree in their descriptions of the mechanism of this contraction.

In presenting evidence relative to the muscular nature of pericapillary cells, Vimtrup (1922), investigating the capillaries in the webs of frog and salamander larvae, describes the state of the Rouget cells during various conditions of the capillary. He describes the transitions from arteriole to capillary in detail, and concludes that the cells are muscular in action.

Tannenberg (1925) observed a sphincter-like mechanism (Kapillarpförtnerzellen) at the point of origin of the capillary from the arteriole. The responsible cells were described as spur-like structures extending into the capillary, and by the use of vital trypan blue he stained cells with proto-

plasmic processes at the capillary origin. These were the sphincter cells.

Bensley and Vimtrup (1928), in investigating the living capillaries of the tongue of the frog and of the surviving nictitating membrane, report that the Rouget cells contract, and are therefore muscular. They list the following characteristics to be used in distinguishing true Rouget cells from other pericapillary cells:

"Contraction spontaneous in origin or resulting from experimental stimulation actually observed in the living animal or in the surviving tissue properly prepared; demonstration of morphological differences in the Rouget cells which are characteristic of muscle cells and not of other mesenchymal elements; demonstration of a definite nerve supply to the cells in question."

Jones (1936), in preparations made from the iris of an albino rabbit, stained by methylene blue injection of the whole animal, describes the pericapillary cells as muscular, and suggests that they are derived from the typical smooth muscle cells of the small arterioles. Figure 1 (page 12), which is taken from his paper, shows the transition in arrangement from small artery to capillary.

Similarly, Zweifach (1937), in describing the structure and reactions of the small blood vessels of Amphibia, says that although typical smooth muscle cells are not found on the true capillaries mentioned before (page 10), they are abundant on the arterio-venous bridges. These are the more direct channels between arteriole and venule. They seem to remain open at all times and have an uninterrupted flow of blood. The muscular cells of these bridges, as shown in Figure 2 (page 12), taken from Zweifach (1937), are peculiar thin cells; when stimulated mechanically the underlying wall is constricted. However, they are apparently not as highly differentiated

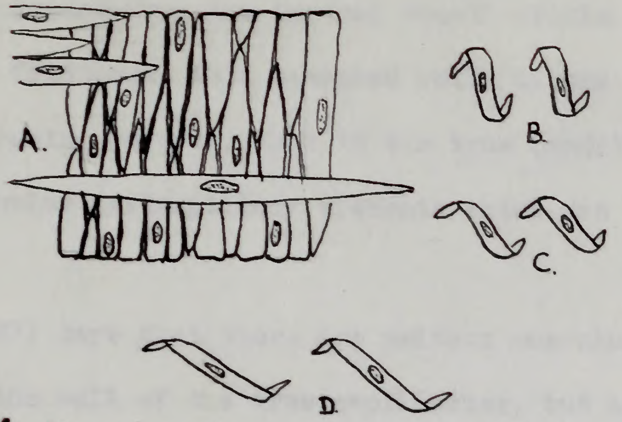

Figure 1. Muscular elements of small blood vessels. A. Small artery, showing longitudinal and circular muscle fibers. B. Arteriole. C. Transitional arrangement. D. Capillary. A stroma unites the margins of neighboring muscle fibers to form the capillary tube. Jones (1936)

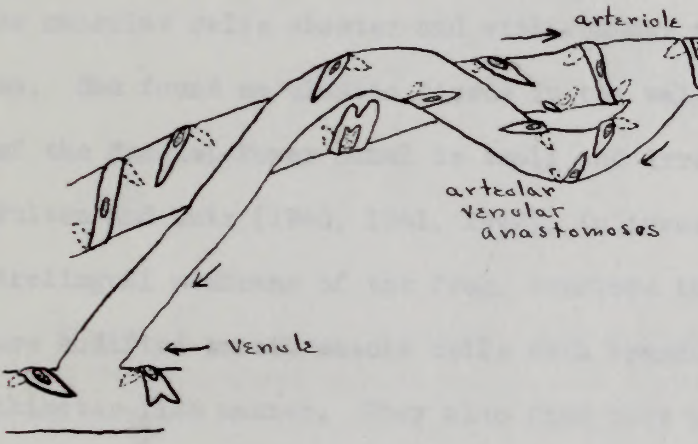

Figure 2. Arterio-venular anastomoses. Muscular cells on proximal segment only. Note branching muscle elements at the point of branching and at curved portion of vessel. Frog, vital methylene blue stain. X300. Zweifach (1937)

nor so effective in constriction as the more typical muscle cells of the arterioles. Figures 3 and 4 (page 14), also taken from Zweifach (1937), show further the change from the typical smooth muscle cell of the arteriole to the peculiar, flattened, thin branched cells of the arterio-venous bridge. Zweifach states again, however, that in the true (endothelial) capillaries there are no muscular pericapillary elements which can contribute to contraction.

Jordan (1937) says that there are neither muscular cells nor connective tissue cells in the wall of the true capillaries, but adds that precapillary arterioles and venules may have a thin layer of muscular tissue.

M. E. Brown (1937) describes muscular Sucquet-Hoyer canals in the tongue of the dog. The wall of these vessels contains clear endothelial-muscular cells, involving a considerable thickening of adventitial and muscular coats, with the muscular cells shorter and with rounder nuclei than those of the arteries. She found no elastic tissue in the wall, and states that the lümen of the Sucquet-Hoyer canal is small and irregular.

Fulton and Lutz (1940, 1941, 1942), in investigating the capillaries of the retrolingual membrane of the frog, conclude that at the capillary origin there are modified smooth muscle cells with branched processes which act in a sphincter-like manner. They also find that neither endothelium nor pericytes contract. From observations on the innervation of the capillaries, they conclude that the smooth musculature of the blood vessels is organized in definite localized motor units.

Whether or not these pericapillary cells (pericytes, Rouget cells) are smooth muscle, it is definitely known that smooth muscle occurs chiefly in the walls of the hollow or tubular viscera. Jordan (1937) classifies the

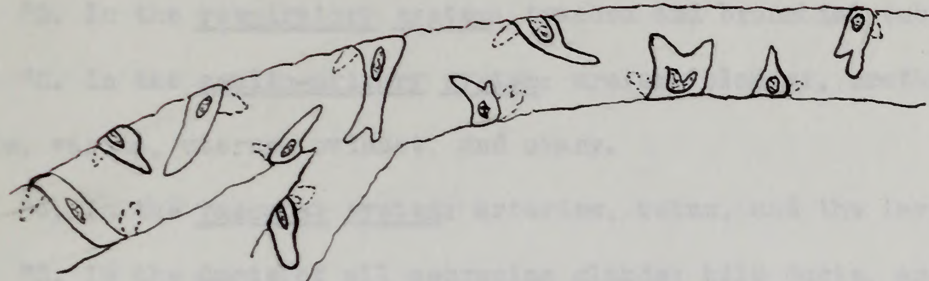

Figure 3. Peculiar flattened muscle cells on an arterial-venal bridge vessel in the frog. Vitally stained with methylene blue. X 370. Zweifach (1937)

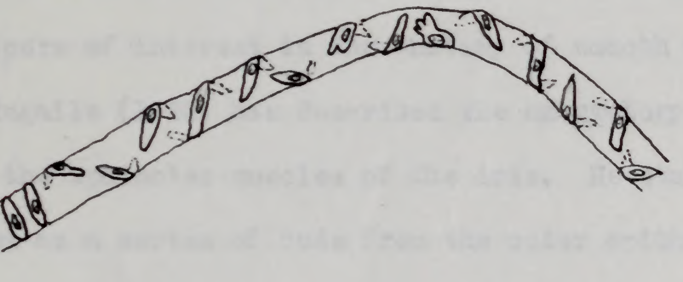

Figure 4. Typical smooth muscle cell on a small arteriole disrupted where the vessel bends sharply. Note branched cell in curved regin. Frog. X 185. Zweifach, (1937).

distribution of smooth muscle as follows:

"1. In the alimentary tract: lower portion of the esophagus, stomach, small and large intestine.

"2. In the respiratory system: trachea and bronchial tubes.

"3. In the genito-urinary system: ureter, bladder, urethra, penis, prostate, vagina, uterus, oviduct, and ovary.

"4. In the vascular system: arteries, veins, and the larger lymphatics.

"5. In the ducts of all secreting glands: bile ducts, and gall bladder, and the ducts of the pancreas, salivary glands, testicle, etc.

"6. It is also found in the capsules of the spleen and lymph nodes, in the skin, and in the intrinsic muscles of the eye.

"Small numbers of branching cells have been described in the walls of the urinary bladder and the large arteries."

Three papers of interest in the anatomy of smooth muscle have appeared recently. Ingalls (1923) has described the embryology and anatomy of the dilator and the sphincter muscles of the iris. He states that the sphincter muscle arises as a series of buds from the outer epithelial cells of the secondary optic vesicle near or at the papillary margin. At term, the developing muscle is still connected with its epithelial source, but later it becomes entirely free, and is finally found as a narrow band of typical smooth muscle embedded in and surrounded by the stroma of the iris. In certain Carnivora (the otter), the muscle is extremely large, but still smooth muscle; in birds, however, the muscle of the sphincter is striated.

The dilator muscle also arises from the same layer of epithelial cells, though later in embryonic life. However, the muscle cells of the dilator remain connected with their epithelial origin, the individual cells being

termed 'epithelial smooth muscle cells.' The large nuclei are excentrically placed in a heavily pigmented cytoplasm so that these areas of these cells form the outer or anterior layer of the pars iridiae retinae. The contractile parts of the cells remain as a thin, non-nucleated layer lying close to the pigment layer of the iris, but not invading the stroma of the iris.

Macklin (1929), in describing the musculature of the bronchi and lungs, follows the suggestion of Baltisberger (1921) that there are two anatomical divisions of the pulmonary musculature; the bronchial musculature, which is related to the airway; and the interstitial musculature, which is scattered through the interstitial tissue and deeper layers of the pleura. He describes the bronchial musculature as a contractile net which embraces the mucosa and the cartilage layer; it is said to be intermingled with a loosely-woven layer of elastic tissue which is arranged mainly circularly in line with the muscle fasciculi, and it may properly be termed 'myoelastic'.

In the smaller, non-cartilaginous branches of the respiratory tree, the muscle is outwardly bounded by an adventitia, rich in elastic tissue, which passes over into the alveoli. Here the muscular elements thin out, and farther on, in the passages of the respiratory division, the elastic membrane becomes broken up by the alveoli of the wall and blends with the muscle to form sphincters for the annexed alveolar sacs and alveoli.

The interstitial musculature had already been divided by Baltisberger (1921) into three parts: (1) the muscle fibers of the interlobular septa; (2) those of the bronchial and vascular sheaths; and (3) those of the visceral pleura. It occurs in the form of a fenestrated membrane, or in the sheaths about the blood vessels, lymph vessels or bronchi, but yet dis-

tinct from the walls of these structures.

Acheson (1938), in describing the smooth muscle of the nictitating membrane of the cat, says that this muscle arises in two sheets from that smooth muscle which lies in a layer of peribulbar fascia which is continuous with the superficial sheets of the rectus muscle. One of these sheets, termed by him the inferior muscle of the nictitating membrane, arises from the fascia overlying the belly of the inferior rectus muscle and passes anteriorly, superficial to the inferior oblique muscle, to split finally into two lamellae, one of which enters the lower eyelid, and one the nictitating membrane. The other sheet of muscle, which he calls the medial muscle, arises from the fascia of the medial rectus muscle and from the trochlear cartilage. It courses anteriorly, finally also splitting, to insert in part in the nictitating membrane and in part to become continuous with the superior palpebral smooth muscle. He says that neither of these muscle sheets extends far into the nictitating membrane, but inserts only in its origin. This is in direct opposition to the statement of Eccles and Magladery (1937) that the smooth muscle of the nictitating membrane extends almost to its free border. Acheson points out that the location of the muscular tissue of the nictitating membrane should be taken into consideration in the interpretation of results by the many physiologists who have recorded contractions of the nictitating membrane.

The function of smooth muscle, in consideration of its location in the walls of all visceral organs, is clearly the production of such changes in size and shape as occur in these organs.

Best and Taylor (1938) point out briefly the differences in the physiology of smooth muscle from that of striated muscle, and list them as follows:

"(a) slower, more sluggish contraction, (b) greater extensibility, (c) the exhibition of a sustained contraction or tonus for long periods, even though separated from the central nervous system, (d) the power of rhythmical contraction, (e) the possession of a double autonomic innervation (parasympathetic and sympathetic), (f) greater sensibility to thermal and chemical influences and to certain types of mechanical stimulation, such as stretching, but a lower excitability to electrical stimulation."

The physiology of the innervated and denervated smooth muscle fibers will be discussed in detail later. However, a brief discussion of the work done by Ferguson (1940) on the physiology of the nerve-free smooth muscle of the chick amnion will be included here. As many authors have shown, it is almost impossible to obtain nerve-free smooth muscle by denervation technique, and so muscle which is embryonically nerve-free was used by the author in order to observe the purely myogenic contractions of smooth muscle. The 10 to 14 day amnion of the chick was shown to exhibit independent rhythmicity and tonus changes in a bath of isotonic saline solution. The optimum temperature was about 41° C; an increase in temperature to 43° caused a loss of rhythmicity and tone which was regained after lowering the temperature; the vitality of the tissue was lost at 46° to 47° C. At 22° to 23° C, the tissue survived for a short period. Mechanical and electrical stimulation were shown to produce a local response, which occasionally led to a wave of contraction spreading from the point of stimulation. The preparation was not shown to be influenced by stretch. The response to drugs is outlined as follows: adrenaline inhibits the muscle; acetylcholine contracts it (this action is inhibited by atropine). In concentrations sufficient to antagonize acetylcholine, atropine has little or no effect on the

amnion muscle. Adrenaline opposes the contraction of the amnion muscle which is caused by cocaine, ephedrine or tyramine. Ephedrine and tyramine are antagonized by cocaine. Contraction is also caused by morphine sulfate and by digalen.

Ferguson postulates, in regard to the response elicited by acetylcholine and adrenaline in the nerve-free smooth muscle, that the ingrowth of nerves may sensitize and reverse the reaction of smooth muscle to these agents.

RELATION BETWEEN SMOOTH MUSCLE AND NERVES.

A. Morphological.

It is best perhaps to begin with a discussion of the several theories concerning the autonomic nervous end-formation, particularly on smooth muscle. Boeke (1940), in his excellent review of the problems of nervous anatomy, discusses the three theories of sympathetic nervous end-formation as follows.

The first school, headed by the eminent neurohistologist Cajal, believes that the sympathetic ganglion cells, wherever located (inside the spinal cord; ganglia of sympathetic trunk; peripheral ganglia) send their processes, the post-ganglionic fibers, directly and independently to their effectors. They say that whether or not the fibers branch and twist to form complicated plexuses, they remain independent. Eventually the fibers reach the effectors (smooth muscle tissue, gland cells, etc.) where they form delicate small end-rings or end nets. Since several authors have described direct anastomoses between the nerve fibers, this theory seems inadequate and further theories have been established. However, physiological evidence, to be presented later, supports this theory.

The second theory states that the 'interstitial cells' (described by Lawrentjew in 1925) are nervous in action, and that they serve as intermediate elements between the strands of nerve and the smooth-muscle cells or other effectors. Cajal himself, in descriptions of the plexuses of Auerbach and Meissner, has described the neurones of these two plexuses, and in addition a third type, consisting of small, multipolar cells with finely vacuolated cytoplasm and numerous short, richly branching varicose processes which interlace or anastomose to form an irregular network. Since the cells lie in the spaces between the bundles of the nervous plexus, Cajal refers to

them as an interfascicular plexus. They appear to contact chiefly the smooth muscle cells; and Cajal therefore suggests that these interstitial elements are influenced by the sympathetic fibers entering the intestinal wall.

Lawrentjew (1925) more than agrees that these interstitial cells are nervous; he suggests that they are the constant syncytial terminal element behind the end-formation of the sympathetic plexus, and from them grow out the endings on the smooth muscle fibers. Van Esveld, Leontowitsch, Schabadasch, and Boeke himself agree that this is so, and that these elements are to be regarded as true parts of the sympathetic end-formation. Van Esveld (1928) conducted experiments on isolated strips of the circular muscle of the cat's intestine which led him to this conclusion. He freed the preparation of all ganglion cells (verified histologically), but found that the plexus of neurofibrillar strands remained intact. With this plexus present, rhythmic contractions of the muscle persisted. He concluded, therefore, that the contraction of the smooth muscle of the intestine was neurogenic, even though the ganglion cells had been removed, and that it must have been the 'interstitial cells' which were the participating nervous elements.

The majority of the observations described above were made with silver-technique. Leeuwe (1937) used methylene blue, and was able to describe more accurately the course of the interstitial elements and their connections. He showed that the interstitial cells arose as distinct elements with branching processes in syncytial continuity from the clusters of ganglion cells of the developing sympathetic plexus. They spread out in the musculature until they reached the muscle cells themselves, at which point neurofibrillae first appear. These intracellular elements pass backwards from the point of origin until they reach the previously fibrillated elements of

the primary plexus. He also showed Nissl bodies in the cytoplasm of the interstitial cells. Leeuwe therefore concludes that the interstitial cells are derived from ganglion cells, and are therefore modified ganglion cells. He shows that they are in syncytial connection with each other and with the true ganglion cells of the sympathetic plexus, and are therefore to be regarded as primitive neuronic elements.

The third theory is that of the 'terminal reticulum', described by Stöhr and others. Adherents to this theory postulate that "the sympathetic ganglion cells are not separate individuals, whose cell processes end freely or in connection with neighboring tissues by means of true nervous synapses, but large syncytial masses whose processes anastomose everywhere to form an inextricable mass throughout the whole body." There are no true nerve-endings to be found; structures described as nerve-endings by many authors are believed to be artifacts caused by faults in the staining technique. The most important idea of the theory is that there are no nerve endings, and that there is merely a gradual transition from a neuro-fibrillar network into a delicate network of a more protoplasmic nature, which is present everywhere in the body. It has been described in all regions of the body, including smooth muscle, glands, where the individual fibers run between the individual gland cells (in those glands possessing contractile elements in their tubules, these elements are supplied by this plexus), connective tissue, and even in the sympathetic ganglia. It is Boeke's opinion that this is the real efferent sympathetic terminal system.

While it is evident that any of these three theories may be the correct one, or that all three may actually be involved, it is probable that the third theory is the least disputed, and the one grounded on the best histo-

logical evidence. However, several authors, including Nonidez (1939), have stated that this terminal reticulum is not nervous tissue, but connective tissue, and have based their statement on its reaction to the characteristic stains for these tissues.

Hofmann (1907), in describing the innervation of the smooth muscle, describes a coarse net of nerves at the point of entrance of the nerve bundle, which becomes finer and finer until it consists only of tiny nerve fibers which run in all directions through the muscle. These fibers run to the muscle fibers, but do not show definite nerve endings. The fibers may penetrate the cell in the neighborhood of the nucleus; if so, however, without ending they go out again from the innervated muscle. He concludes that the nerve endings observed in incompletely stained preparations are artificial products of these nerve fibers. He could draw no conclusions as to the continuity of the net itself, especially as to the connections between the different fibers.

Tiegs (1924), in his studies on plain muscle, describes a "true nerve network", to which is ascribed the diffuse distribution of the impulse to smooth muscle. In the muscles of the frog's stomach, he describes two nerve fibers terminating on each muscle cell. Both lie within the fiber; the first is a large, quite prominent branched structure, spreading over the whole nucleus and at times apparently wound around it; the second is a very small and slightly branched end plate which ends not on the nucleus but in the granular material close to it. He admits that some cells were found which showed either one or the other, but not both, of these endings, but he ascribes this to the 'capriciousness' of the methylene blue technique, and concludes that every muscle fiber in a plain muscle system has a double innervation.

In 1925, Tiegs described the nerve net of plain muscle, saying that it

is always developed in intimate association with smooth muscle, but never in connection with striated muscle tissue. Nerve fibers arise from the nerve net, and terminate directly on the muscle fiber.

Lawrentjew (1925), in his paper describing the interstitial cells in smooth muscle, confesses that he originally believed them to be connective tissue cells, but after using a modified Bielschowsky technique which demonstrated neurofibrillae in these cells he was forced to change his views. He describes the cells of the sympathetic plexus (which is found in the walls of the digestive canal in addition to the typical plexuses of Auerbach and Meissner) as "Spindelformig oder dreiechigen Zellen mit feiner langer, nach verschiedenen Richtungen verlaufenden Fortsätzen", that is, the typical interstitial cells of Cajal. In these cells he has traced neurofibrillae "deren Verlauf sich leicht bis zu den motorischen Endigungen in der glatten Muskelfasern verfolgen lässt." The neurofibrillae are seen to penetrate into the cells themselves, and lie so close to the nucleus that an indentation (Einbüchtung) is to be noted. He concludes that "die interstitiellen Zellen sind das letzte Glied dieser Leitungsbahn; ihre Fortsätze begleiten die Neurofibrillen bis zu den Endverzweigungen und Endigungen in den glatten Muskelfasern". A later article (Lawrentjew, 1926) repeats these findings.

Evans (1926) states that there is usually, if not always, a double nerve supply to smooth muscle. The nerve fibers are generally non-medullated, and often exhibit varicosities which are regarded as ganglion cells. The terminal nerve fibrils form a fine plexus on the surface of the muscle cells and terminate in small end knobs, one to each cell.

Hill (1927) reports that, in addition to the plexuses of Auerbach and Meissner, there exists in the gut wall as in all organs innervated by the

autonomic system a complex feltwork formed by the interlacing of peculiar cells, the interstitial cells of Cajal. In the gut wall she finds that these cells are in constant relationship with the enteric plexus. Her drawings make it clear that these cells are quite separate units, variable in form, the cell body being oblique or triangular to fusiform, and with a small amount of protoplasm. The nucleus is oval, with no nucleolus and with scattered chromatin granules. The cell body sends off two or more varicose processes which interlace with other processes to form an irregular feltwork. She has traced these processes directly to endings on the smooth muscle cells, and has also observed the extremely delicate fibrillae given off from these endings which penetrate into the substance of the muscle fiber. She adds, however, that she has never seen neurofibrillae in these interstitial cells, and therefore can make no conclusions as to the nervous nature of the interstitial cells. The work of van Esveld (1928) has already been described (page 21).

In 1932 Boeke published a paper which dealt with the innervation of the smooth muscle fibers of the arrectores spinarum of the hedgehog, in which he gives a general description of the innervation of smooth muscle. He refers to the work of Hill, van Esveld, Lawrentjew and Stöhr, and concludes from these and from his own work that the nerve fibers end within the muscle cells. He also mentions the work previously done on the interstitial cells, and states that the 'interstitial elements' described by Lawrentjew are very similar to that which other authors have described as a perivascular nerve plexus. He concludes that the interstitial reticulum is the true efferent sympathetic terminal system. Figure 5 (page 26), which is taken from this paper, is offered by Boeke as proof of the statement that every muscle fiber receives

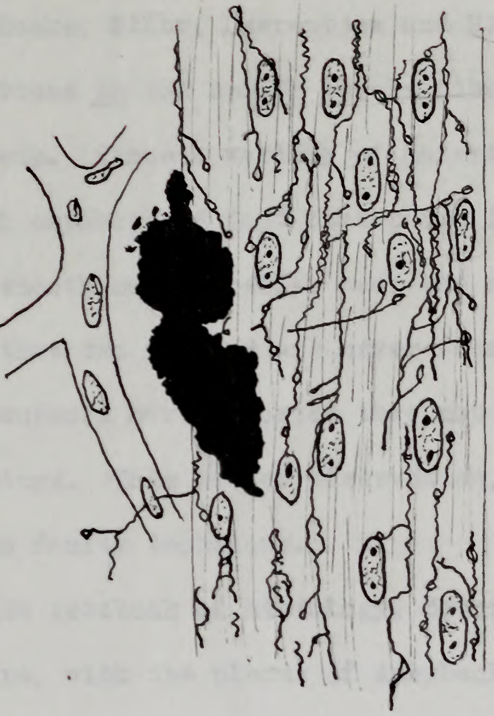

Figure 5. Nervous plexus and endings in the muscularis ciliaris of the eye of a monkey, Macacus, longitudinal section, highly modified. Bielschowsky method, section 10 µ. Boeke (1932)

a nerve ending.

Cannon (1933), in his description of the chemical mediators of the autonomic nerve impulses, reviews first the theories of nerve terminations. He reports the work of Boeke, Stöhr, Lawrentjew and Hill, in which were described nerve terminations in the smooth muscle fiber ending in a fine reticulum near the nucleus. Since a variety of material was used, he concludes that it is a well established fact that nerve impulses are delivered to the interior of the smooth muscle cells, near the nucleus.

He goes on to say that two neurons are never found on the same muscular element, and that many authors have reported that not all smooth muscle cells possess nerve endings. This latter observation, as has already been mentioned, may be due to faulty technique.

Cajal (1933), in his textbook of histology, describes the smooth musculature of the intestine, with the plexus of Auerbach (the autonomic center of the intestine) between the circular and longitudinal muscle fibers. The nerve fibers of this plexus give off collateral and terminal nerve branches around the cells.

In his discussion of nerve endings in smooth muscle, he recalls the work of Arnstein (1887), who was the first to point out true terminations of nerve fibrils in the smooth muscle of the frog's bladder, using methylene blue. Cajal, using the same stain, reported nerve endings in the intestine and bladder.

In accordance with other authors, he describes three nerve plexuses: one, fundamental or supramuscular, which is situated on the surface of the muscle; an intermediate plexus, found between the smooth muscle cells; and

a third plexus, intramuscular or interfibrillar, composed of fine independent ramified cells which occupy the cement of union of the contractile corpuscles. The fibers of this last plexus represent the axis-cylinders which course between the muscle cells, ramify two or three times in a right angle, with delicate rosary-like terminal endings in contractile protoplasm. He adds that commonly each fiber ramifies into an extensive arborization whose branches run parallel to the interstices of the fibrocells with which they make contact.

Cannon and Rosenblueth (1937) review the work on the nerve endings in smooth muscle. They state that, although the earlier work claimed endings on the surface of the muscle cells, more recent work, and particularly that of Boeke, Stöhr, Lawrentjew and Hill, seems to show that the nerve endings are intracellular. They refer to the work of Tiegs which describes two nerve filaments ending in single muscle cells, and state that it is more probable that only one ending is present. It remains to be decided, particularly in tissues which possess double innervation, whether this ending can serve both parasympathetic and sympathetic control, or whether (which is more likely) fibers of both sorts are distributed to different cells. They add that another point disputed at present is that involving the actual number of smooth-muscle cells directly innervated. It seems that, in spite of careful technique, such authors as Stöhr report that only one cell in a hundred is innervated; while other authors (Boeke) report that in certain areas there are sufficient nerve terminals to supply each muscle fiber. The presence of a chemical mediator, as they point out, is sufficient to produce contraction of previously denervated cells; from this they conclude that an individual nerve supply for each contractile element is not

necessary.

Eccles and Magladery (1937a), in physiological experiments on the excitation and response of smooth muscle, conclude that each all-or-nothing unit of this muscle (a unit probably being identical with a single fiber) is directly innervated by several motor nerve fibers. Further work (1937b) leads them to a confirmation of this theory.

Stöhr (1938), in a paper reviewing the microscopic innervation of the blood vessels, describes the peripheral innervation by autonomic elements as dependent on a nervous terminal reticulum. He describes this 'vegetative' nerve end apparatus as a syncytially-built net which is supplied in part with nuclei. The net contains nervous plasma strands which surround the cells of the innervated tissue, appearing as a delicate veil around these cells. The finest branches of this reticulum appear to be united continuously with the plasm of the innervated cells.

Bozler (1939b), in a review of the physiology of smooth muscle, states that smooth muscle supplied by motor nerves, unlike visceral muscle, which he describes as syncytial, consists of a great number of muscular motor-units or individual cells. Hinsey (1939) reviews much of the work just described on the innervation of smooth muscle elements.

Klopp (1940), recalling the fact that the majority of histological evidence favors a sparseness of innervation which results in one nerve fiber to more than one smooth muscle cell, adds that motor units consisting of single smooth muscle fibers probably do not exist.

While the work just reviewed deals with the innervation of smooth muscle in general, with no reference to its location or function in the body, the majority of articles deal with the smooth musculature of definite areas

of the body. Since it is conceivable (and many authors insist) that either the musculature itself or the type of nerve termination, or both, may vary in the different regions of the body, the discussion to follow will be grouped according to the smooth muscle found in the various regions of the body, in accordance with the outline given by Nowland (1929).

a. The vascular musculature. In view of the volume of work published in recent years which deals with the nature of the contractile mechanism of the capillaries (which has already been briefly reviewed; page 8), and in view of the importance of vascular reactions to the well-being of the individual, a great deal of the anatomical and physiological descriptions of the autonomic system which have been published within the last twenty years, deal, if not entirely, at least at great length with the innervation of the blood vessels.

Pulmonary. As far back as 1904, Brodie and Dixon concluded, from physiological investigations, that the pulmonary arterioles do not possess a vasomotor nerve supply, although they admit the presence of nerve endings. However, Larsell (1921), in discussing nerve terminations in the lung of the rabbit, describes motor terminations in the muscular coat of the pulmonary vessels. He states that there are relatively large nerve trunks which accompany the larger pulmonary arteries. There are no ganglion cells to be found, and he admits that the source of these trunks is not definitely known. He states that the smaller branches of the pulmonary artery lie close to small bronchi, and are innervated from the nerve plexus around these pulmonary units. In his description of the microscopic appearance of this innervation, he says that the nerve fibers wind around the blood vessels, giving off individual fibers at more or less irregular intervals. These fibers run

roughly parallel with the artery, then turn nearly at right angles to the longitudinal axis of the latter, and divide into several main branches. One of these rami usually runs back along the artery, proximally, from the point of primary division of the fiber; another runs distally from this point. These larger branches give off numerous slender varicosed fibers which in turn subdivide. The final fibers pass between the smooth muscle bands of the tunica media, and give off twigs which terminate in relation to the smooth muscle cells. Figure 6 (page 32), which is taken from this paper, is offered by Larsell to illustrate the innervation of the muscle cells of the tunica media. He says that these terminal twigs end as small knobs, apparently on the surface of the smooth muscle cell, and usually near the nucleus.

As to the pulmonary capillaries, he mentions briefly that some preparations show a delicate nerve plexus on these vessels with no indication of nerve cells. The musculature of the tunica media of the veins is apparently innervated in the same manner as is that of the arteries.

A paper published by Larsell in 1922, dealing further with the lung, describes a nerve plexus which is present about the vascular tubes, and repeats his statement that the nerve fibers terminate in relation to the smooth muscle cells of the tunica media of these vessels.

Later, Larsell and Dow (1933) continue the discussion of the innervation of the lung, and describe a periarterial plexus composed of large nerve trunks around the base of the pulmonary artery and its larger branches. These trunks diminish in size with the vessels they supply, and finally become individual fibers around the smaller arterial branches. Some of these fibers go directly to the parenchyma, and others through arterioles to the parenchyma; once in the parenchyma, the smaller filaments follow the course

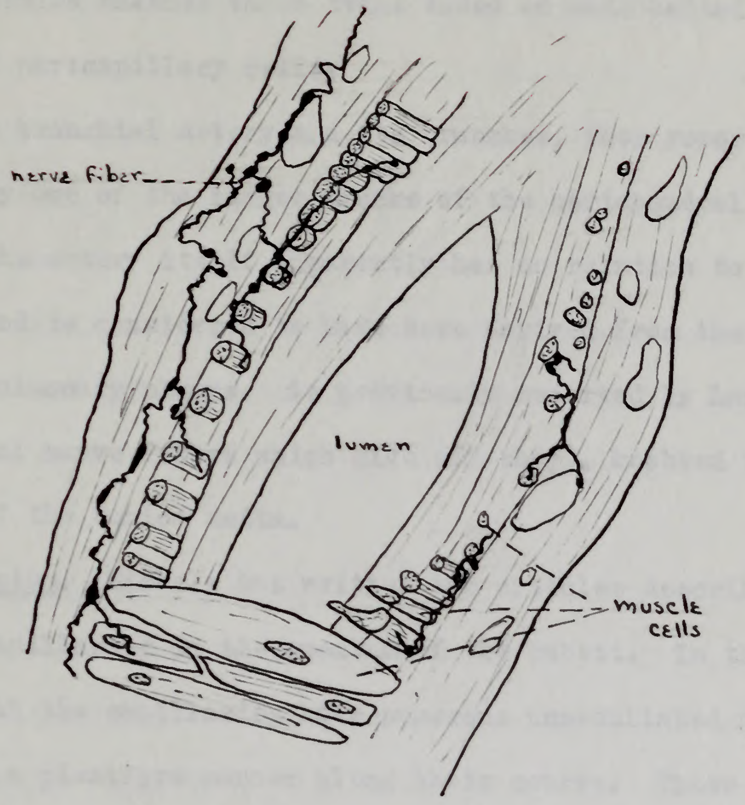

Figure 6. Arteriole from pulmonary artery, showing a single nervefiber in the adventitia which gives off short branches which terminate in relation to the individual smooth muscle cells of the tunica media. Methylene blue with aurantia counterstain. 25 µ. X 880. Larsell (1921).

of the capillaries about the alveolar ducts and air sacs, giving off at intervals short terminal twigs which end on the capillary walls. They could not determine whether these twigs ended on endothelial cells or on other types of pericapillary cells.

The bronchial artery and its branches, they report, are usually accompanied by one of the larger trunks of the perichondral plexus. The plexus around the artery itself apparently has no relation to the accompanying trunk, and is considered to have been derived from the sympathetic component of the pulmonary plexus. As previously reported by Larsell, the plexus shows individual nerve fibers which give off short, knobbed twigs to the smooth muscle of the tunica media.

Omentum. Michels has written two articles describing the innervation of the capillaries in the omentum of the rabbit. In the first (1935) he shows that the capillaries have numerous unmedullated nerve fibers distributed in a plexiform manner along their course. These capillary nerves show an intimate contact with the capillaries, touching them, running parallel to them, crossing over and under them, and in general accompanying them, but never show any knob-like endings on the endothelium. Since he found that many capillaries have no nerves accompanying them, and since endings are never found, he concludes that capillary innervation may be accomplished as a physiological unit, the mechanism being a closed nervous syncytium, which functions at its contact points with the endothelium. It will be remembered that this is the theory professed by Stöhr and Boeke. However, Michels believes that their 'terminal reticulum' is a connective tissue network of reticular fibers, and is in no way nervous.

He defines the term 'plexus omentalis' as the plasmodial conducting

system in the omentum of the rabbit. In the capillary bed it consists of a very widely-meshed plexus of extremely narrow (1 μ) homogeneous-staining plasmodial strands which intermittently (200 to 300μ) contain oval Schwannian nuclei either in the interior of the strands or on their periphery. There are slight expansions of the plexus strands at the nuclei, and at the points of its branching. He found no evidence for the sympathetic innervation of Rouget cells.

His second article (published in 1936) reports more specifically on the nature of the pericytes in the omentum. He was able to find no evidence for the muscular character or the sympathetic innervation of pericapillary cells.

Ductus Arteriosus. An interesting set of papers on the innervation of the ductus arteriosus has been published by J. D. Boyd. In his first paper (1937) he describes two sets of fibers reaching the ductus. The first are thick fibers, which terminate in the adventitia chiefly, but partly in the media. These are receptor fibers, and therefore not autonomic; their function in the closure of the ductus at term is discussed. The second fibers are thin, and end mainly in the media, in relation to the muscle fibers.

In his second paper (1941) he describes the oblique direction of the muscle fibers in the wall of the ductus, and postulates that the motor fibers described above initiate and maintain the sphincter-like contraction of the muscle.

Coronary vessels and vessels leading from the heart. Glaser (1914), in an article describing generally the nerves of the vessel walls, describes three nerve nets in the aorta. The first is adventitial; the second lies in the meshwork between adventitia and media, and from it single nerve fibers and

meshes are to be recognized between muscle fibers or between connective tissue bundles. The third nerve net is spread out in the inner part of the muscular coat, and single fibers of this plexus appear to penetrate into the intima.

Nonidez (1939) describes the distribution of the cardiac nerves. He says that branches from the cardiac ganglia supply the coronaries; the parasympathetic fibers being vasoconstrictors, and the sympathetic, vasodilators. In the atrium and the interatrial septum, bundles of nerves break up, and their fibers supply elaborate plexuses to the externa of the arteries, from which numerous fibers reach the surface of the media to end in contact with the smooth muscle fibers.

Katz and Jochim (1939) describe the origin of the fibers to the coronary vessels as follows: the vagi supply only cholinergic vasodilator fibers, and there is no evidence of cholinergic vasoconstrictors. The stellate ganglion sends both adrenergic vasodilator and adrenergic vasoconstrictor fibers to the coronary vessels.

The vessels of the skeletal muscles. Krogh, Harrup and Rehberg (1922) describe on a physiological basis the innervation of the vessels in the hind leg of the frog. They find the arteries and capillaries in this region to be kept in a state of variable tonic contraction through the sympathetic fibers. The arteries and many of the capillaries were shown to respond to posterior root stimulation with dilation, and some of the arteries and capillaries appear to be innervated by anterior root fibers, which when stimulated cause slight dilation.

Since chemical stimulation of a slight area in the frog's web causes dilation over the whole web independently of the central nervous system,

they conclude that an extensive nervous network is responsible for the reaction.

Hinsey (1928) finds that the thoracolumbar sympathetic fibers are distributed to blood vessels containing smooth muscle and to a small portion of the capillary bed.

Tiegs (1932), in his study of the innervation of the intercostal, leg and tail muscles of a lizard, found that after degeneration of the spinal cord and somatic nerves the only unmyelinated nerves (necessarily sympathetic) were found in close association with the large or small blood vessels, and probably never in the plexus of fibers which was independent of these.

The tongue. Krogh (1920), in his studies on the innervation of the blood vessels in the tongue of the frog, found nerve endings in the walls of the capillaries and arterioles which he said must have a double function, being both sensory and dilatory.

Barksdale (1926), studying the capillary innervation, found that the capillaries in the frog's tongue were innervated by contiguity rather than continuity with the nerve fibrils; on the other hand, he presents the fact that the arterioles are innervated by continuity with the nerve fibrils, and not by contiguity.

Most recently, there is the work of Fulton and Lutz, done at Boston University, which deals with the theory of smooth muscle motor units in the small blood vessels of the frog's retrolingual membrane. In their first paper (1940) they describe modified smooth muscle cells at the point of capillary origin which are shown to regulate the capillary blood flow in a sphincter-like manner without the aid of the supplying vessel. They show a dual innervation of these cells in many cases, for brief faradic stimulation of the small vasomotor nerves produces first dilation of the small blood

vessels which is later followed by constriction of the same vessels. In many cases, however, certain fibers produce only one type of response to any strength of the stimulus. Their histological preparations show an anatomically continuous loosely-meshed non-myelinated nerve plexus continuous with the perivascular plexus; they add that it is possible that dilator and constrictor fibers may at times become separated.

Since faradic stimulation of small nerves produced responses restricted to small vascular areas, they conclude that the plexus described above is physiologically discontinuous. Stimulation of any one of several nerves in an area often produced the same response; therefore the idea of a smooth muscle motor unit is suggested to them.

Their report published in 1941 repeats the evidence given above, which leads them to the conclusion that "the anatomically continuous perivascular nerve plexus, which is rich on the arterioles and precapillaries but sparse or lacking on the capillaries, is physiologically discontinuous."

Central nervous system. S. L. Clark has written a series of papers dealing with the innervation of the blood vessels of the central nervous system. In the first (1928) he describes motor nerve fibers to the smooth muscles of the vessels of the choroid plexus of the fourth ventricle. In the second (1929) he states that the intrinsic arteries of the medulla and spinal cord are supplied with two kinds of fibers, namely, coarse fibers probably medullated and sensory, and fine unmyelinated fibers, which are derived from bundles of similar fibers in the pial arteries, and which end in relation to the smooth muscle cells of the media. He was not able to find nerves on the capillaries or veins of the medulla and spinal cord. In his third article (1934) Clark reports that the pial blood vessels possess many

small nerve bundles and plexuses composed of a few myelinated and many unmyelinated fibers. Both of these branch, and terminate, some in the adventitia of the veins and arteries, some among the smooth muscle cells of the media of the arteries, and some on the pia mater between vessels. He found only meager evidence for capillary innervation.

Capillaries of the iris. Jones (1936) has reported extensively on the structure and innervation of the iris. His theories regarding the muscularity of the pericapillary cells have already been described (page 11). He points out that his muscular pericapillary cells are not the cells described by Rouget; and, by reason of his staining technique, believes that the Rouget cells are nervous in function. In his own words, "the Rouget cell is a unit of the neurilemma together with the accompanying nervous filament and its branches, as these appear in an exceptional position. It is presumably not contractile, but is motor to the other element present, whatever that is, or to a third element for the existence of which there is no evidence at all."

General. The majority of authors who have published references to the innervation of the blood vessels do not restrict their findings to the vessels of any particular area, but describe the innervation of these vessels in general. The work of these authors will now be reviewed.

Woollard (1926), in his article describing the innervation of blood vessels, reports that the vasoconstrictor fibers are purely sympathetic, and that, in supplying the vessels of the limbs, they travel with the peripheral nerves. Section of these nerves causes vasodilation; their stimulation brings about vasoconstriction. The sensory nerves are capable of conducting impulses antidromically, causing vasodilation.

He says that the bundles of nerves which are found associated with

the blood vessels are largely of non-medullated fibers; they cross, divide and rejoin as branches in the adventitia of the aorta at all levels, some superficial and some near the media. They descend from the aorta along the iliac arteries, forming an interlacing plexus of non-medullated nerve fibers, which give off individual fibers to the media where they form an abundant mesh of fine fibers within the muscular coat.

As this mode of innervation becomes reduced and disappears, a second type appears. This involves the supply of small twigs from the adjacent nerve trunk. It is predominately derived from medullated nerves, there being three to four medullated fibers for each myelin-free fiber. The non-medullated fibers, when found, can always be traced to the diffuse net which surrounds the muscular coat of these arteries as well as the whole vascular system.

The muscular plexus is composed of the finest non-medullated fibers. It usually presents a beaded form, and occasionally bears slight oval expansions. It ramifies through all layers of the muscular coat, and forms a true net, with dividing fibers which rejoin and divide again, so that conduction can take place in all directions. From the individual strands in the net tiny side branches are given off, and these end in small swellings after an extremely short course. He reports that the terminations are always pericellular and not intracellular.

He then continues his description by saying that the capillaries receive a nervous investment which belongs to the sympathetic system, and which is generally a continuation of the arterial investment but may be derived from the nerve trunk itself. Definite endings in the capillary are not found. In addition, each capillary is generally accompanied by two

nerves which run on each side of it, with cross anastomoses between them at frequent intervals. They form no terminal apparatus on the endothelium, but are associated with the Rouget cells; the latter lie in the plexus without contact with it, or may actually appear to be in contact with it.

Krogh (1929) describes the innervation of the capillaries in much the same manner as Woollard (page 39), but states that very fine fibrils, penetrating the Rouget cells have been found.

Boeke (1932), in his article on the innervation of smooth muscle previously referred to (page 25), states that in the connective tissue of the choroidea and accompanying the blood vessels of the human eye, a plexus of very fine amyelinated nerve fibers is seen to run in small bundles with intercalated nuclei similar to the interstitial elements. This plexus encircles the blood vessels and capillaries wherever they are seen.

He has found in the walls of arteries from various sources an intricate network of strands and neurofibrillae which covers the entire muscular coat and extends between the muscle layers and between the outer cells. It is found everywhere, on and in the media of the smaller and larger arteries. He demonstrated its connections with the sympathetic fibers, and observed its extending to the muscle cells and continuing into an extremely delicate network inside the muscle cells.

Clark and Clark (1934a), in describing the richly-muscular arteriovenous anastomoses in the rabbit's ear, found a rich nerve supply which they stated was apparently related to the partial independence in contraction rhythms of an A-V-A from its supplying artery.

M. E. Brown (1937), in her article on the Sucquet-Hoyer canals in the tongue of the dog, describes two types of innervation in these vessels,

namely a myelinated sensory type, and a thinner, probably non-myelinated fiber, which she believes to be concerned with vasoconstriction, since it ends on the surface of the modified media of the Sucquet-Hoyer canal.

Moon (1938), in his book on shock and related capillary phenomena, describes capillary innervation as follows: "Most of the vasoconstrictor nerves belong to the sympathetic system....Their terminal fibers form a veritable network of anastomosing branches in the muscular coat of the smaller arteries. In some areas and in some animals non-medullated fibers have been shown accompanying the capillaries, and capillary constriction following sympathetic stimulation has been described." Moon states that "So far as I know the ending of a nerve fiber in a cell or cells of the capillary wall has not yet been found."

Stöhr (1938) cites the work of others, and expresses his belief that the nervous terminal reticulum found in the vessel walls is a true and uniform syncytial picture.

b. The dermal and ectodermal musculature. I was unable to find any reference in the more recent literature which concerned itself with the innervation of the smooth muscle of the skin.

c. The urogenital musculature. Gruber (1938) reviews extensively the work done to the above date on the autonomic innervation of the genito-urinary system. For a complete bibliography of this less recent work, the reader should consult this paper.

He states that the urogenital musculature is supplied mainly with fibers from the sympathetic division and the fibers from the sacral division of the parasympathetic system. He then goes on to discuss the innervation of the various parts of the urogenital system, his work being fonnded on a

physiological basis in most instances.

Musculus spiralis papillae. The spiral muscle of the papilla is apparently innervated by parasympathetic as well as by sympathetic fibers.

Kidney capsule. The capsule is innervated by vasomotor nerve fibers of the autonomic nervous system, but the smooth muscle fibers of the capsule are not innervated directly by either inhibitor or motor nerve fibers.

Ureter. Nerve trunks from the plexus renalis, the plexus spermaticus and the plexus hypogastricus send nerve fibers along the course of the arteries in the ureter, forming a rich 'ground-plexus' in the adventitia. From this meshwork, nerve fibers pass into the tunica muscularis along its length. No satisfactory nerve endings have been found on the smooth muscle of the ureter and it is estimated that there is one nerve fiber for every fifty smooth muscle fibers. The fibers of the ureter are shown to be inhibitor and motor sympathetic and motor parasympathetic fibers.

In connection with the flow of fluid from the ureter to the bladder, Stewart (1937) reports that this flow is controlled by a sphincter which possesses its own intrinsic innervation. Langworthy and Murphy (1939) describe the innervation of the ureter, and state that nerve fibers run along muscle bundles and appear to give rise to motor endings, the endings being sparse in the preparation.

Urinary bladder. Gruber (1938) adds that the pelvic nerve is generally agreed to be the main motor nerve of the bladder. Postganglionic fibers of this nerve travel short distances as fiber groups and are abundant in the adventitia where they give off fibers to the mucosa and the submucosa. In the muscle, they are less abundant than in the adventitia. They divide into

the finest terminal network to supply the smooth muscle cells. The best reports indicate that there is one nerve fiber for every twenty-five to one hundred muscle cells and that the nerve fibers do not penetrate the nucleus of the cell.

Langworthy has published two articles which deal with sensory nerve endings in the smooth muscle of the urinary bladder, but which mention also the motor innervation of this organ. In the first of these articles, written in collaboration with Kleyntjens (1937), a rich plexus of myelinated and unmyelinated fibers is described in the region just beneath the peritoneum and outside the muscles. The myelinated fibers end in sensory endings, and will not be discussed here. The fine unmyelinated fiber is believed to be motor, and it divides into a number of branches which run parallel to the muscle layer. These branches end in fine granules on the muscle. It is impossible to define the exact relationship of the ending to the nucleus and cytoplasm of the muscle cell. The endings may occur on muscle groups running in different directions and are not confined to one sheet of fibers. The endings are numerous, but they cannot state that each individual smooth muscle cell receives a motor ending.

The blood vessels are shown to form a rich capillary network in the bladder. The larger arteries are accompanied by nerve trunks, and nerve fibers run on smaller vessels, ramifying on their walls.

The second article (Langworthy and Murphy, 1939) adds that the capillaries are supplied by fine unmyelinated fibers which show small punctate enlargements along their course, and in which are found loops and festoons encircling the capillaries.

Urethra. Gruber (1938) continues with a description of the innervation

of the urethra. In the male, bundles of nerve fibers are found between the longitudinal and circular musculature, and also in the submucosa. The urethra receives autonomic fibers from three sources; the hypogastric, which contracts the arteries and may also contract the smooth muscle of the genital skin; the pudic, which probably supplies vasomotor and motor and inhibitor fibers to the smooth muscle; and the pelvic, which after stimulation causes a relaxation of the urethra which involves both the circular and the longitudinal musculature.

Prostate gland. The smooth muscle around the acini of this gland, which contracts during ejaculation, is supplied with motor fibers from the pelvic and hypogastric nerves.

Seminal vesicles. Enveloping the musculature is a fibrous coat containing many small ganglia. Nerve fibers and plexuses of nerve fibers are found in the adventitia, muscularis and submucosa. The musculature is innervated by the hypogastric nerves and the lumbar nerves.

Vas deferens. A nerve plexus is found in the adventitia, in the submucosa and in the muscular coat of the seminal ducts. The hypogastric nerve is the motor nerve.

Clitoris and labia minora. Efferent motor and vasoconstrictor nerve fibers and efferent inhibitor and vasodilator fibers to these organs are derived from the hypogastric and the internal pudic nerves.

Ovary. This organ is innervated from the ovarian plexus or hypogastric plexus and from the plexus sacralis. A plexus of nerve fibers is described around the blood vessels in the organ. There are also believed to be fibers in the follicles. These are thought to supply the smooth muscle stroma of these structures, and are probably not concerned with their secretion.

Fallopian tube. The greatest bulk of this organ consists of smooth muscle which undergoes periodic rhythmical contractions. The nerves are derived from the inferior mesenteric ganglion and the hypogastric nerves. The nerve fibers enter the adventitia, forming there a dense network from which finer fibers pass to the muscular layer where they form a very fine intramuscular nerve plexus. The plexuses appear not only about the longitudinal and circular muscle cells, but also surround the muscle of the blood vessels. In all cases the efferent nerve fibers appear to end as bare nerve endings, with no special end structures.

Vagina. The longitudinal muscle fibers of this organ are continuous with the superficial muscular fibers of the uterus. The two layers, longitudinal and circular, of the muscle are not distinctly separable from each other, but are connected by oblique fibers which pass from the one to the other. Many fine bundles of nerve fibers are found in the adventitia of the vagina, and these form a nerve plexus around the vaginal wall. Fibers from this plexus pass to the muscular coat to form an intramuscular plexus. The sympathetic innervation of the vagina is motor in all animals; there may be an inhibitory sympathetic nerve supply which may predominate over the motor effect. There may also be a motor parasympathetic supply.

Uterus. There is a dense nerve plexus in the adventitia, with some of the fibers penetrating the muscular layer to form an intramuscular network. The fibers usually run parallel to the blood vessels and some penetrate the smooth muscle cells of the blood vessels and uterus to form a fine fibrillar network. As far as can be determined, these fibers end in bare nerve endings.

Davis, in 1933, published a review which dealt extensively with the innervation of the uterus. For a bibliography of the earlier work in this

field, the reader is referred to his paper. He himself has found nerve fibers, both myelinated and unmyelinated, scattered through the uterine substance. They arise from nerve bundles of considerable size which are situated in the periphery of the uterus. In the body of the uterus the nerve fibers are found lying between, and parallel with, the smooth muscle fibers, and surrounding the capillary blood vessels. He was not able to demonstrate any specific type of nerve endings.

Reynolds (1937), in dealing with the nature of uterine contractility, finds that conclusive evidence shows that nervous factors do not provide the integrating basis for oestrus uterine activity. In the first place, he states that in the rabbit there is no extensive intrauterine innervation; in the second place, transplanted denervated uteri respond in a characteristic manner to the motility-stimulating action of oestrin. He suggests that changes in the intrinsic metabolism of the uterus are responsible for the myometrium-activating action of oestrin.

Bozler (1938a), in an article which gives evidence for the syncytium of smooth muscle in the walls of the uterus, finds in his experiments that a diffuse plexus of any kind, although it may be present, is not responsible for the responses of uterine muscle. He assumes that the conduction of any impulse takes place through the muscular syncytium.

W. H. Brown and Hirsch (1941), reporting on the intrinsic nerves of the immature uterus, have found that nerves, accompanied by large blood vessels, pass from the cervical ganglia on the lateral sides of the cervix into the wall of the uterus. The large nerve trunks extend parallel to the long axis of the uterus and lie deep within the myometrium. Small branches extend from these trunks to the endometrium, without entering the epithelial lining cells.

They form an intricate network of plexus in the lamina propria of the cervical canal. The significance of the close anatomical connection between the nerves and blood vessels within the uterine wall is not clear, though a similar relation exists between the nerves and blood vessels in other tissues of the body. Nerve endings in the vascular wall could not be demonstrated.

d & e. The musculature of the gut. Carpenter, in 1918, published an article which described the sensory nerve endings in the muscular coat of the stomach and the small intestine. In this paper he mentioned observing terminal structures, whose source could not be determined, in the form of skeins and nets, both composed of fine fibrils with numerous varicosities, which occurred in the longitudinal muscle coat of the cardiac stomach of the cat. These endings he said were connected with unmyelinated varicose nerves. Although he does not say that these are motor nerve endings, from the description given and the conclusions of other authors it is probable that these are motor nerve endings.

Langley (1921), in his description of the connections of the enteric nerve cells, presumes that the vagus fibers are the preganglionic neurons involved, and that these end in the enteric nerve cells. This he deduces from the fact that the postganglionic fibers are not paralyzed by nicotine. The complex reactions of the gut, especially peristalsis, and histological findings disprove the theory that only one nerve cell is on the course of an impulse to the gut, as in other autonomic tissues, and show that sensory nerve cells are present in the enteric system; also that peristalsis is a coördinated reflex.

The nerve strands which the vagus sends to the greater part of the small intestine are small, and the enteric nerve cells supplied by them are in

hundred thousands, if not millions. The vagus cannot then run to them all, and it is probable therefore that the later-formed enteric cells lose direct connection with the vagus, though remaining connected with it through cells which may be regarded as mother cells. This is illustrated in Figure 7 (page 49) which is taken from Langley's paper. In this diagram, there are no sensory cells, and peristalsis would be the result of stimulation of the vagus cells by the contents of the gut and by the distension of the gut wall. It will be noticed that the connections of the vagus with the gut differ from that of other autonomic nerves in that it is tri-neuronic rather than bi-neuronic.

Lawrentjew (1925), in the article already referred to (page 24) which deals with the nature of the interstitial cells, mentions that the plexus formed by these cells is present in the walls of the digestive canal in addition to the typical nerve plexuses of Auerbach and Meissner. His description of these plexuses does not differ from that of other authors.

Hill (1927) goes into considerable detail in her description of the enteric plexus. She says that the myenteric and submucous plexuses are alike in that they consist of groups of nerve cells connected together by intrinsic and extrinsic fiber-bundles. The extrinsic (vagal) fibers end in relation to the nerve cells and the processes of the latter convey the impulse within to muscle and glands or to nerve cells in other plexuses.

In their detailed characters, she finds that the two plexuses differ. In Meissner's plexus the ganglia are thin and flattened and the cells are closely grouped together in a single layer; in Auerbach's plexus the ganglia are thicker and more massive and the cells lie superimposed on each other.

She describes ganglion cells of two types; those of the first type are

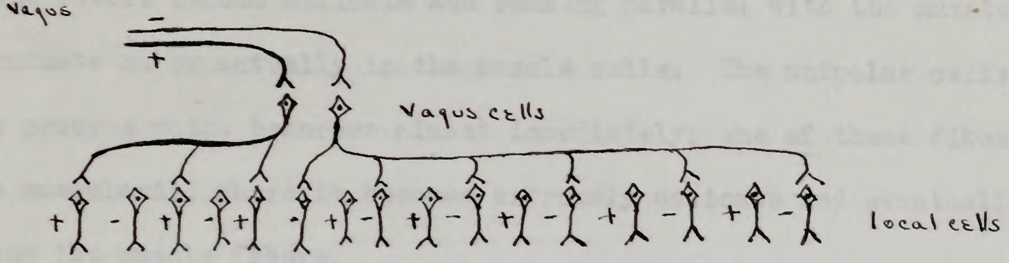

Figure 7. Diagram illustrating the connections of the Vagus nerve with the enteric nerve cells of the intestine. +, motor fibers; −, inhibitor fibers. Langley (1922.)

sensory, and will not be considered here. The cells of type II, however, are motor, and she divides them into three classes, based on their structure. The multipolar cells are observed to terminate in relation to the muscle coat, and the cells of it; the bipolar cells run without division to the muscle coat, running as thick varicose fibers among the muscle cells. Ultimately, these fibers become delicate and running parallel with the muscle fibers terminate on or actually in the muscle cells. The unipolar cells send out one process which branches almost immediately; one of these fibers goes to the muscularis, where it becomes extremely delicate and eventually disappears among the muscle fibers.

Her description of the interstitial cells in the gut wall has already been referred to (page 24); it will be remembered that she reports that these cells are in constant relationship with the enteric plexuses.

She reports having found motor nerve endings in smooth muscle, the endings being derived from the neurons of the 'intramuscular plexus', which she finds lying in parallel bundles between the smooth muscle cells. She states that these fibers are found to terminate around the muscle cells in characteristic networks, and that varicose swellings of the fibrils on these cells probably give rise to extremely delicate fibrillae which penetrate into the substance of the muscle fibers.

In 1928, van Esveld published a paper which dealt extensively with the nervous elements in the wall of the cat's intestine. He says that the circular musculature contains a certain number of scattered ganglion cells of the type of Auerbach's plexus, which are present singly or united in smaller or larger groups. The majority of these cells are said to be laid down in the part of the musculature where the mesentery is fixed to the intestine. He

divides the ganglion cells of Auerbach's plexus into two classes, as did Hill. In addition to these ganglion cells and the plexus connected with them there is also a nervous syncytium connected with the interstitial cells, which he says are nervous in character; this syncytium he states could be important for the intestinal movements, but he adds that the exact function of the syncytium is still to be ascertained.

Schabadasch (1930a), in discussing the innervation of the musculature of the cat's stomach, states that the most important intramural nerves are represented by the plexus subserosus, the plexus myentericus and the plexus submucosus. The largest part of the extramural nerve fibers (from the vagus) enters into the myenteric plexus, spreading out in this and giving off branches to the other two plexuses. The geometrical form of the plexus myentericus changes three times in the course of the stomach; at the fundus, in the body and at the pylorus, and is characterized by a definite and constant type of network. The largest ganglia and the most important count of nerve cells are found at the pylorus and the pyloric canal; the number of the same decreases considerably in the direction of the fundus.

The subserous and submucous plexuses do not seem to innervate the smooth muscle cells, and it is therefore not necessary to consider them at any great length. However, he has noted connections between the submucous and myenteric plexuses which are accomplished by a considerable number of nerve fibers.

A second article by Schabadasch (1930b) deals with the innervation of the cat's intestine. In this organ he describes five plexuses: a) plexus subserosus; b) plexus myentericus (externus, Auerbach); c) plexus muscularis profundus (Cajal, Drasch); d) plexus entericus internus (Henle); and e) plexus submucosus (Meissner). The first of these does not innervate smooth muscle,

excepting that of the blood vessels in its effector region, and can therefore be disregarded in this paper. The second and third plexuses listed above (myenteric and muscularis profundus) are found in the muscular coat of both the small and large intestine; they are separated on the basis of number of ganglion cells, size of the ganglia, and size and form of the network.

Through both of these plexuses the muscle layers of the digestive canal are said to be penetrated by an immense number of nerve bundles. The motor endings in the intestinal wall are recognized incompletely. The fourth plexus listed above (plexus entericus internus) is in reality part of the submucous plexus; it is found next to the plexus myentericus in the boundary between the tunica submucosa and the layer of circular muscle of the intestinal tract. It is quite similar to Auerbach's plexus.

The submucous plexus is also to be disregarded here, since it innervates the tunica submucosa and not the smooth muscle.

Irwin (1931), in describing the anatomy of Auerbach's plexus, emphasizes the variability in nerve cells and ganglia along the tract, and refers to the nerves which anastomose with the plexus. He used the methylene blue technique in staining sections from the gastrointestinal tract of the guinea pig. He defines Auerbach's plexus as "a continuous ganglionated nerve plexus supplying the smooth muscle cells of the outer longitudinal and inner circular muscular coats of the gastrointestinal tract and the sphincters arising in connection with them." The plexus lies between the muscular coats, adhering to the outer coat in the oesophagus and stomach, and to the inner coat from the pylorus to the anus. In its macroscopic appearance, it is described as a "continuous delicate and irregular mesh, with thickenings where the component branches meet." The thickenings correspond to the ganglia, which can be seen

to differ in size and shape. In its microscopic appearance, it is noticed that the ganglia stain well, with the nerve cells clearly outlined and the nuclear structure well indicated. The interganglionic fibers are evident, and the intraganglionics are clearly seen ramifying between the neurons. The neurilemmal nuclei, which appear light purplish-blue in contrast to the deep blue fibers, are found on inter- and intraganglionic fibers, and ramifications of the interganglionics to the nerve cells. The nerve bundles in the adventitia of the blood vessels stain, as does the plexus in the muscular coat of the vessels. Meissner's plexus is also seen.

Irwin has found Auerbach's plexus in all areas of the gastrointestinal tract, from the level of the oesophagus at which smooth muscle first appears to the level of the internal anal sphincter, which it supplies. He was unable to find connections between Auerbach's and Meissner's plexus.

Irwin, in discussing the terminations of the interganglionic fibers, states that they give off small branches, usually perpendicular to the main trunk, which rebranch immediately and apparently are distributed solely to either the inner or to the outer muscle coats. The smaller branches lie in the muscular coat parallel to the muscle fibers, and the individual fibers terminate in several irregular beaded endings. These endings are usually two to three times as long as the muscle fibers, and they are devoid of the neurilemmar covering, which terminates when the fiber divides into its endings. Each individual muscle fiber does not seem to have an individual nerve ending, each nerve ending apparently innervating two or more muscle fibers by direct contact, and many muscle fibers are apparently devoid of any contact with nerve endings.

In 1931, McSwiney published a review of the nerve supply and movements

of the stomach, and the function of the vagus and the sympathetic nerves with relation to the motility of the organ. His description of the vagal and sympathetic innervation is entirely gross anatomy, and will not be considered here. His description of the enteric plexuses is taken from that of Hill, which has already been referred to (page 24). He states, however, that the innervation of the gut is of the same character as that of other organs innervated by the autonomic nervous system; the vagal fibers are connector fibers which connect with many cells by means of collaterals, and these cells are the motor cells to that part of the gut musculature which belongs to the vagus system. He does not describe nerve endings.

Garry (1934), in describing the movements of the large intestine, discusses briefly the intrinsic innervation of this organ. He states that Auerbach's plexus is developed better caudad than craniad in the large bowel. The more powerful the muscle coats, the closer is the mesh of the myenteric plexus and the more numerous the ganglion cells. Meissner's plexus is also present in the colon.

Li, in 1940, published a very comprehensive paper which dealt with the intramural innervation of the small intestine. He describes the circular muscle of this organ as consisting of two layers, a thick external one and a thin internal layer. The external layer is supplied by the tertiary branches of Auerbach's plexus and a cell-net of the interstitial cells of Cajal; the inner layer is richly provided with nerve fibers which are said to be of local origin, since they represent the protoplasmic processes of a rather primitive type of interstitial cell of Cajal. These cells are well-developed in the space between the circular and longitudinal muscle and in the external layer of the circular muscles. He describes definite relation-

ships between the muscular cells and the three nervous elements, the interstitial cells, the nerve fiber strands, and the ganglion cells of Auerbach's plexus. He states that the interstitial cells of Cajal are probably ganglion cells.

In more detail, he describes the innervation of the various regions of circular muscle. In the inner layer of circular muscle, he finds that individual nerve cells from Meissner's plexus never seem to supply muscle fibers directly; however, he states that these cells form a fine reticulum which is closely associated with Cajal's interstitial cells; as such, they would innervate the smooth muscle cells. In addition to this network, nearly every cell of the inner circular layer is said to be surrounded by nerve fibers, the protoplasmic processes of a type of interstitial cell of Cajal, which branch infrequently and anastomose among themselves. In the outer layer of the circular muscle, he has observed that a few varicose processes of the nerve net appear to terminate as end knobs on the muscle fiber; this may be an artifact, however. In the longitudinal muscle layer, Auerbach's plexus is found, and in addition, Cajal's interstitial cells.

From this description, he concludes that there are two types of nerve net; (1), located in the inner layer of the circular muscle and composed of rather primitive interstitial cells; and (2), located between the circular and longitudinal muscles and within the outer layer of the circular muscle, and composed of well-developed interstitial cells. From the primitive nature of the nervous elements in the inner layer of the circular muscle, Li suggests that this layer may be considered as neuromuscular in nature, and may have definite relations to irritibility, conduction and rhythmic contraction.

In concluding, he states that the interstitial cells of Cajal are to be considered as the end-point of the enteric system and that these can carry on ordinary activity in the absence of vagus and sympathetic fibers, and the plexuses of Auerbach and Meissner.

f. Vision. Best and Taylor (1938) describe briefly the structure and functions of the iris. It is generally agreed that its musculature is composed of two sets of smooth muscle fibers, the constrictor and the dilator pupillae. Ingalls (1923), in a paper already referred to (page 15) has described the embryology of these muscles. The control of the muscle of the iris is purely reflex; the efferent fibers for the constrictor reflex belong to the parasympathetic system, and those for the dilation reflex, to the sympathetic system. Light falling on the retina of either eye causes both pupils to constrict, the constrictor pupillae being excited while the dilator pupillae is inhibited. In dark or dim light, the tone of the latter is increased while the constrictor is inhibited, and the pupil dilates.

Boeke (1933), in describing the iris muscles in reptiles and birds (it will be remembered that in the latter case the iris muscles are striated), states that in the ciliary body he found an anastomosing plexus of nerve fibers with nerve endings in two regions, the first, around connective tissue cells, and the second, a fine anastomosing net of neurofibrillar bundles running between and sending fine branches into the muscle fibers. Here they end as intraprotoplasmic rings near the nucleus.

Jones (1932), in the article already referred to (page 11) in which he described particularly the capillaries of the iris of the albino rabbit, stained with methylene blue, reports also his observations on the innervation of the cells of the iris muscles. He states that over the greater part of

the iris the dilator muscles are visible as a series of radiating pillars, within several of which can be seen an arborization of very minute neurofibrillae of finer texture than the fibers in relation to ordinary smooth muscle. Over the entire iris is a fine nerve network arising from the larger nerves. The meshes of this net are gathered together near the pupillary margin to form arches from which branches return between the fibers of the sphincter and, turning at right angles, enter the muscle fibers and ramify within them. The nerves supplying the capillaries arise from the same network, and in some cases a nerve can be traced to its source in the same fiber which supplies one of the muscles. He found no nerve cells in the iris, and says that the nearest cells are those in the scleroid coat.

S. L. Clark (1937), in reporting on the innervation of the intrinsic muscles of the cat's eye, says that in the muscles of the iris and the ciliary body of the normal eye there are nerve fibers branching about smooth muscle cells, their final fibers being parallel to the long axis of the muscle fibers. Where the fibers branch there is usually a triangular expansion showing neurofibrillar structure. In the course of the smaller subdivisions there are varicosities which vary in size and shape and upon the ends of the fibers are seen small knobs or rings. He finds that there are certainly enough terminal nerve fibers for the separate innervation of each muscle cell.

He did not observe intraprotoplasmic endings, but reports that the knobbed endings or varicosities are closely related to the muscle fibers. In many instances, he found that the nerve fibers did not stop on a particular muscle cell but seemed to have an expansion or varicosity which rested on the surface of the cell and extended beyond to repeat this on other muscle fibers, or to end in a knob near another fiber. This knob or varicosity is frequently,

though not invariably, near the nucleus of the muscle fiber. He found no evidence for a terminal anastomosing net.

He concludes that each ciliary muscle fiber is closely related to at least one nerve fiber in its terminal portion, but says that this does not imply any similarity to other smooth muscle innervation.

The nictitating membrane, because of its accessibility, has been used by many physiologists in recording contractions of smooth muscle. The anatomy of the smooth musculature in the nictitating membrane has already been described (page 17), and I have been unable to find references which deal with its intrinsic mechanism. However, Rosenblueth and Bard, in 1932, published a paper which dealt with its extrinsic innervation and functions. They observed that the retraction of the nictitating membrane is caused by the smooth musculature, which is supplied by the sympathetic system. The protrusion of the nictitating membrane is caused by striated muscle in the external rectus, which is innervated by the sixth cranial nerve. They state that "the nictitating membrane presents the paradox that smooth muscle innervated by the sympathetic (the retractor mechanism) has striated muscle as an antagonist (the protrusion mechanism). The different functions of the two mechanisms are in harmony with their different histo- and physiological arrangement: retraction (smooth muscle) is usually tonic and stable, whereas protrusion (striated muscle) occurs only sporadically."

In his classification, Nowland (1929) omitted two important areas in which smooth muscle is found, namely, in the respiratory system and in the ducts of the glands.

g. The respiratory musculature. In 1921, Larsell published the first of a series of articles which dealt with the innervation of the lung. He has

found that the musculature of the bronchi is a direct continuation of that of the trachea, and that the same pattern of innervation must hold for the bronchial smooth muscle and that of their branches as for the trachea. In the walls of the larger bronchi, where plates of cartilage are present, the muscle occurs in bands; in the smaller subdivisions, where there are no cartilaginous plates, the muscle is formed in a continuous sheet. There are no muscle fibers found distal to the alveolar ducts, but there is a sphincter-like band at the extremities of these ducts, surrounding the openings into the atria. These various muscle bands have a very rich innervation, which is motor in nature, since the nerve fibers are smaller than the sensory ones and do not have a myelin sheath.

Most of the nerve fibers of the smooth muscle bands are postganglionic; when they reach these structures numerous slender varicosed rami are formed which run parallel to the bands between the smaller bundles of muscle cells. At intervals they give off short twigs which terminate as small knobs on the muscle cells. Fine nerve fibers may also leave the main nerve trunk, in which case they pass to the muscle bands to end as described. Larsell states that the innervation of the muscles of the bronchi is so very rich as to permit two origins; indeed, it is much richer than the processes of the intrapulmonary ganglia would seem to show.

Regionally, the nerve bundles follow the bronchi in diminishing size to the broncheoli and to the alveolar ducts, giving off branches which are distributed to the smooth muscle bands around these smaller air passages.. The most distal location of the motor nerve fibers is that at which the small sphincter-like muscle bands are found, at the openings of the atria from the alveolar ducts. Slender varicosed fibers have been shown to run along the

muscle cells in these bands and to terminate in relation to them.

In 1921 Larsell and Mason conducted degeneration experiments involving the relation of the vagus nerve to the nerve terminations in the rabbit's lung. They report that the vagus supplies the preganglionic fibers to the intrapulmonary ganglion cells, and that after degeneration of the vagus the number of fibers in the bronchial musculature and in the walls of the pulmonary blood vessels is affected very little, if at all.

A third article by Larsell appeared in 1922. Here he describes in particular the plexuses of the mammalian lung. The extrinsic nerves of the lung, in addition to the vagus, are supplied by the anterior and posterior pulmonary plexus. In the lung, near the hilum, they become segregated into three groups of plexus, namely: (1) a bronchial group; (2) a vascular group; and (3) a plexus which is distributed to the pleura pulmonaris. The second has already been referred to (page 31); the third does not innervate smooth muscle.

The bronchial plexuses, he says, are two in number, an extrachondral and a subchondral plexus. The extrachondral plexus, which is composed of relatively large nerve trunks that anastomose to form a plexus of longitudinal mesh about the bronchi, is seen to lie between the cartilaginous plates of the bronchi and the lung parenchyma. It contains many myelinated fibers and numerous clusters of ganglion cells. The subchondral plexus, which lies between the layer of cartilaginous plates and the layer of the bronchial muscle, is composed of some ganglion cells and smaller nerve trunks which consist of unmyelinated fibers. The myelinated fibers pass to sensory terminations in the epithelium and in the smooth muscle bands; the unmyelinated, to motor terminations in the smooth muscle of the bronchi. In the smaller bronchi, whose

walls do not contain cartilaginous plates, the two plexuses blend into one, which retains many of the characteristic features of the component plexuses.

Macklin (1929), in reviewing the musculature of the bronchi and lungs, defines its location in the same way as Larsell (1921; page 58). He mentions the anatomical evidence which shows nerve fibers destined for the bronchial tree; numerous ganglia in the bronchial wall from which arise numerous postganglionic fibers for the muscle cells; and the endings of these fibers on the muscle cells. He then goes on to correlate this innervation with the bronchial movements, saying that he believes that the bronchial muscle is actively engaged, and not passive, in the respiratory process. The stimulus to expiration is probably the attainment of a certain stage of distension in the lungs; inspiration, in other words, initiates impulses which, traveling over the vagus, influence the respiratory center and inhibit the further outflow of inspiratory impulses. This same impulse may, by means of other connections, stimulate the center in the vagus nucleus which causes the bronchial muscle to contract.

In 1933, Larsell and Dow published a paper dealing with the innervation of the human lung. They describe, in the same manner as Larsell (1922, page 60), the periarterial and peribronchial plexuses, and the two subdivisions of the latter. The postganglionic fibers of these plexuses divide into smaller and smaller bundles and reach eventually the bands of smooth muscle, in which they break up into individual fibers which give off terminal twigs to the smooth muscle cells. Such efferent fibers are said to pass to the smooth muscle of all divisions of the bronchial tree, as far distad as the opening of the alveolar duct into the atrium.

h. The musculature of the ducts of glands. Again, there have been no recent publications dealing with the innervation of this smooth muscle.

B. Physiological.

The greatest volume of work done on the physiology of smooth muscle has undoubtedly been done by Cannon and Rosenblueth and their colleagues. The monograph published by Cannon and Rosenblueth in 1937 reviews the work which had been done to that date, and contains a complete bibliography covering the field of autonomic neuro-effector systems.

They begin their book with a discussion of the organization of the autonomic system, noting that the sympathetic division is clearly organized for a distribution of the nerve impulses. In association with this is the innervation of the adrenal medulla by sympathetic preganglionic fibers. When the medulla is stimulated, adrenine is liberated; this has been shown to mimic the action of the sympathetic system, and thus when it is distributed through the body by the blood stream it brings about the coöperation of most of the effectors. This is obviously important for the protection of the individual during emergencies, and for the preservation of steady states in the organism.

As is well known, many organs in the body (heart, gastro-intestinal tract, bladder and blood vessels in some parts) are innervated from both the sympathetic and one of the parasympathetic divisions. When both the systems innervate one organ the two are generally opposed in their effects. Not all smooth muscle is supplied with nerves from both divisions, however, for only sympathetic fibers innervate the nictitating membrane of the cat, the uterus, the pilomotor muscles, and probably the majority of the blood vessels.

They outline the steps in the development of evidence for chemical mediation of nerve impulses, describing the experiments which led to the theory that, when a sympathetic or parasympathetic nerve is stimulated, a substance is liberated into the blood stream to be carried to other effectors of

the same system, where it may effect a similar result. The parasympathetic mediator has been shown to be acetylcholine; that which is liberated in sympathetic stimulation was first believed to be adrenalin, but has since been shown to be a closely related chemical, which Cannon and Bacq first termed sympathin. When a paralyzing agent, such as atropine or ergotoxine, prevents the effecting of autonomic stimulation, it has been shown that the mediator is produced, but is in some manner unable to reach the responsive mechanism in the cell.

In general, organs or tissues which serve as sources of either parasympathomimetic or sympathomimetic substance will also serve as indicators of the presence of these substances in blood perfusate coming from another tissue. In the parasympathetic system, the indicators and the effects of the mediator on them are as follows: contraction of the stomach and intestines, constriction of the bronchioles, narrowing of the pupil, and dilation of the blood vessels in various parts of the body, particularly the external genitalia, the face and the salivary glands. The indicators of the presence of the sympathetic substance are: the blood vessels and pilomotor muscles, the nictitating membrane, intestine, retractor penis, uterus, spleen and iris; the effects are opposed to those of the parasympathetic system.

Many authors have noted that there is a distinct difference in the stability of sympathin and acetylcholine, the latter being destroyed almost immediately after its formation while the former may be active for ten minutes or more. Cannon and Rosenblueth point out that this is related significantly to the neural organization of the sympathetic and parasympathetic systems. The sympathetic system, as has already been mentioned, is arranged to work as a unit, and the provision for diffuse discharge of sympathetic impulses, found

in the overlapping of preganglionic fibers in their courses up and down the sympathetic chains, is reinforced by the simultaneous discharge of adrenaline and sympathin, which have additive effects. The parasympathetic neurons, on the other hand, are arranged for specific responses in separate organs, and the extreme lability of acetylcholine prevents the distribution of impulses to other effectors through the presence of a mediator in the blood stream. Incidentally, it has been shown that eserine inhibits the rapid destruction of acetylcholine, and augments the acetylcholine contractions.

It has been shown by many workers that the sympathomimetic substance is not adrenaline, but a different compound closely related to it, sympathin. Among the differences listed is the fact that sympathin is unlike adrenaline in having two separable effects. From this fact, Cannon and Rosenblueth have postulated two kinds of sympathin, excitatory (sympathin E) and inhibitory (sympathin I). They have found that adrenalin acts to stimulate some smooth muscle cells and to inhibit others. However, there is no reason for assuming two different types of smooth muscle cells. There must be present inside some muscle cells an agent which modifies adrenalin to make the fibers shorten, and in others, an agent which modifies adrenalin to make them lengthen. Similarly, the agent, which is like adrenalin, released by sympathetic impulses may produce two effects, and again the differentiating agent or condition is supposed to be located in the affected cells. An analysis of the action of both adrenalin and nerve impulses on smooth muscle cells led Rosenblueth to the view that a substance A (from the outside; adrenalin) or M (a local product) unites in the cell with a hypothetical substance which may be either E (excitatory) or I (inhibitory). The combination, when nerves afe stimulated, would be ME in a contracting and MI in a relaxing muscle. There-

fore, sympathin may be defined as "the chemical mediator of sympathetic nerve impulses, ME or MI, which in the cell induces the typical response, contraction or relaxation, and which escaping from affected cells into the blood stream, induces typical responses in remote organs controlled by the sympathetic system."

Many electrophysiological investigations into the behavior of the autonomic effectors have been made. It has been shown that many smooth muscles respond mechanically with typical twitches when single shocks are applied to their excitatory nerve supply. These are the 'single-volley' muscles; among them are those of the nictitating membrane, the pilomotors and the bladder. Other neuromuscular systems, however, fail to respond after single shocks. These are the 'multiple-volley' muscles, and include the musculature of the uterus. The single-volley systems also show electric responses, as well as mechanical reactions, after a single stimulus; the multiple-volley systems have not been shown to yield detectable electrograms after single shocks.

In the response of single-volley muscles to single nerve volleys, at least three components, I, II and III, can be distinguished in the electrograms recorded. The first component, I, is a monophasic spike which precedes contraction. Its latency varies in different neuromuscular systems and with the site of application of the stimulating electrode on the nerve. It has been shown that its polarity, that is, its electrical sign in the records, depends only on the orientation of the muscle cells with respect to the recording electrodes. The magnitude of I is a function of the number of active muscle cells interposed between the recording leads; thus it may be concluded that the electrograms of smooth muscle sum in series.

Component II is more complex and variable than I. It is usually poly-

phasic, and its latency is difficult to determine, for I usually merges into II without a distinct break. Component III is a prolonged monophasic deviation which is roughly coincident with the mechanical response. It is difficult to say whether III precedes contraction or not.

In the response of smooth muscle to repetitive stimulation, the main difference between single-volley and multiple-volley systems appears to be the presence of I in the former and its absence in the latter.

Certain observations have been made on the action of a number of substances on the electromyograms. It has been shown that when the nictitating membrane contracts in response to adrenine, I and II are decreased or disappear altogether. According to the membrane theory of excitation, the spike potentials of skeletal muscle are due to a depolarization of a polarized interface. Analogously, I may be due to this phenomenon at the surface of the muscle cells, and its magnitude would then depend on the degree of polarization of this surface. Adrenalin, then, in depressing I, may be considered to partly or completely depolarize the cells.

Ergotoxine, on the other hand, paralyzes the contraction of smooth muscle in response to sympathetic nerve impulses. In the nictitating membrane, which goes into a marked, prolonged contracture after a small dose of ergotoxine, both electrical and mechanical responses are abolished; in the pilomotors, which do not contract after ergotoxine, it has been shown that nerve impulses still call forth characteristic I and II potentials.

Cocaine increases the mechanical responses to sympathetic nerve stimulation, but depresses the components I and II. The reverse is true of the actions of yohimbine and 933F on the nictitating membrane and the pilomotors.

Cannon and Rosenblueth (1937) then combine the two theories of trans-

mission, and list the theoretical links in the excitatory chain as follows:

(1) Nerve action potential
↓
(2) Liberation of mediator inside the innervated cells.
↓
(3) Combination of the mediator with the receptive substance
↓
I ← (4) Depolarization of the surface of the key cells, and
(5) diffusion of mediator and the compound (sympathin E)
↓
II? ← (6) Depolarization of adjacent non-innervated cells, and
(7) penetration by mediator;
↓
II? ← (8) further combination with the receptive substances in these cells.
↓
III ← (9) Depolarization of other interfaces and contraction.

With regard to the excitability of the autonomic effectors, it has been pointed out that there are two types of gradation of response in the effectors. Any nerve impulse follows the all-or-none law, and changes in the response are due to the number of impulses delivered per unit time. Variations in this factor may occur in two ways; a smaller or greater number of nerve fibers may discharge at a constant frequency (spatial variation) or a constant number of nerve elements may deliver impulses at different frequencies (temporal variation). Both modes of gradation may occur in physiological responses. Experiments show that temporal and spatial summation, in smooth muscle, are quantitatively interchangeable; that is, the responses are a function of the number of nerve impulses which reach the effector per unit time, and are independent of the number of nerve fibers concerned. The direct and indirect response of smooth muscle is in agreement with the theory of innervation of only some of the cells.

It remained to be determined, however, whether the smooth muscle cells themselves were electrically excitable, or whether the electric stimulus conducted along the nerve fiber liberated a mediator, which was responsible

for the response of the effector. The available evidence seems to indicate that smooth muscle is electrically inexcitable. Three methods were used in determining this; first, the excitability of the two tissues was determined. Results were not conclusive, however, and so a second method was used. In these experiments the muscle was stimulated directly, and stimuli sufficient to produce visible injury of the tissue near the electrodes failed to evoke contraction. This suggests that the muscle was electrically inexcitable. As a check, a third group of experiments was performed. Here the muscle was denervated, either by section of the nerves or by the blocking action of such a drug as ergotoxine. In these tests results indicated that the smooth musculature is electrically inexcitable. Therefore, it seems probable that the double action of electrical stimulus and mediator is involved in the response of smooth muscle to induction shocks.

In considering the pharmacology of the autonomic neuro-effector system, the steps occuring during neuromuscular transmission are given as: "1) preganglionic nerve impulse→ 2) postganglionic nerve impulse → 3) chemical mediator→ 4) receptive substance→ 5) specific response." Of the substances which mimic the effects of autonomic nerve impulses, adrenalin and acetylcholine, nicotine and the quaternary ammonium bases, and potassium salts act between steps 3 and 4, while barium salts and histamine are believed to act at step 5.

Of the substances which act to block autonomic responses, curare, nicotine, acetylcholine (on adrenergic nerves) and relatively strong potassium salts act between steps 1 and 2; atropine blocks parasympathetic responses after step 3; ergotoxine and piperidinomethylbenzodioxane (933F) block the sympathetic responses also after step 3; 933F may also block at the sympa-

thetic ganglia.

Eserine, cocaine, ergotoxine, sodium bicarbonate, amino acids, calcium salts, and thyroxine have been shown to augment various actions of the autonomic nervous system.

Denervation experiments seem to indicate that depriving structures of their nerve supply renders them more readily responsive to their natural stimuli. Various reasons for this are discussed, but no conclusions are made.

This brief discussion of Cannon and Rosenblueth's book allows six general classifications of the work done on the physiology of the autonomic nervous system as it applies to this paper, and the review which follows of the most recent work in this field will be discussed under these six headings.

A. Dual innervation. As has already been mentioned, it is a well-established fact that the majority of the smooth musculature is under the control of both the parasympathetic and the sympathetic nervous systems. Bozler (1936), in describing the inhibitory action of vasodilator nerves, discusses the regulation of the tone of blood vessels by two antagonistic kinds of nerve fibers, vasoconstrictor and vasodilator. Sympathetic nerve impulses are vasoconstrictor and keep the muscular walls of the vessels in a condition of moderate contraction; dilation may result from a cessation or diminution of vasoconstrictor activity, but may also be produced by the action of specific inhibitory nerves.

Bozler (1940) describes both excitatory and inhibitory effects in the muscle of the non-pregnant cat's uterus; he finds that these results are not easily explained by the assumption of specific excitatory and inhibitory nerve fibers, and suggests that excitation and inhibition represent two phases of the action of the same nerve impulses, inhibition diminishing the excitability of the muscle and blocking muscular contraction, and excitation being

a result of the setting up of muscular impulses by nerve impulses.

Oppenheimer (1938), in describing the autonomic control of the cat's retractor penis muscle, points out that this muscle represents an easily-obtained unit for experimentation in the field of dual innervation. He states that its innervation is derived from the sympathetic system almost exclusively by way of the abdominal sympathetic chain, and from the parasympathetic via the visceral (pelvic) branches of the first two or three sacral nerves. In his paper, evidence is presented which shows that the sympathetic fibers are adrenergic, and the parasympathetic, cholinergic. These two indicators act independently, and one does not prevent the action of the other. The effect of simultaneous discharge of both neural elements is the resultant of the two opposite influences.

B. Chemical mediation. G. L. Brown (1937) reviews the literature concerning the transmission at nerve endings by acetylcholine, and lists the distribution of cholinergic nerves as including not only the postganglionic fibers of the bulbo-sacral outflow, but also the following postganglionics of the sympathetic system: cholinergic fibers in the sympathetic supply to the stomach, and in the hypogastric nerve supplying the uterus; occasionally, a few cholinergic fibers are found in the sympathetic supply to the nictitating membrane; and cholinergic vasodilators, in the intestines of some animals.

Burn (1938) describes cholinergic sympathetic vasodilators in the muscles of the dog. In the dog's intestine the sympathetic vasodilators are adrenergic.

Youmans (1938) points out the similarity of the effects of adrenalin and inhibitory sympathin on the motility of the dog's intestine.

Lissák, in 1939, reported that the results of stimulating isolated

nerves of the cat and the frog. He concludes from his results that cholinergic nerves liberate acetylcholine, and adrenergic nerves, an adrenalin-like substance. The mixed nerves of course liberate both substances.

The following quotation is taken from Hinsey's review of the physiology of the autonomic system (1939).

"The terms sympathetic and parasympathetic as applied to the autonomic nervous system must be used on an anatomical rather than a functional basis. The terms that have been used for a functional classification are cholinergic and adrenergic. The postganglionic fibers in the sympathetic outflow may be adrenergic or cholinergic; those in the parasympathetic, cholinergic and possibly adrenergic. The preganglionic fibers in both divisions are said to be cholinergic as are the somatic motor ones. Some of the fibers in posterior roots are cholinergic, that is, the ones which produce vasodilation."

Cannon and Lissák (1939) give evidence which leads them to infer that adrenalin is distributed through adrenergic neurons. The results are consistent with the view that adrenalin is liberated at the endings of adrenergic fibers, and that sympathin, which escapes from the stimulated region into the blood stream, is this adrenalin modified in the affected cells.

Hebb (1940) reports that the most usual effect of electrical stimulation of the stellate ganglion on the isolated perfused lung is the production of marked bronchoconstriction. The response is shown to be qualitatively similar to that produced by the injection of acetylcholine, for both are potentiated by eserine, temporarily depressed by suitable doses of adrenalin, and suppressed by ergotoxine and by atropine, either entirely or in part.

From experiments conducted on the actions of adrenalin and acetylcholine on the denervated iris of the cat and monkey, Bender and Weinstein (1940)

suggest that excess of acetylcholine stimulates the secretion of adrenalin and that excess of adrenalin stimulates the liberation of a cholinergic-like substance in the body.

Nachmansohn (1941) reports that his experiments suggest to him that acetylcholine does not act specifically as a 'synaptic transmitter' of nerve impulses to effector organs, but is intrinsically connected with the electrical changes occurring everywhere at the surface of neurons during activity.

C. Electrical phenomena. Eccles and Magladery (1937b), in describing the rhythmical responses of smooth muscle, show that a motor nerve volley to the smooth muscle of the nictitating membrane sets up an initial complex potential wave which is followed by later rhythmic potential waves, each preceeding a corresponding step in the muscular contraction. These waves are composed of short, spike-like units, and each is followed by a refractory state in the muscle units. Thus they conclude that these rhythmic waves are produced by the periodic discharge of all-or-nothing impulses in the units of the smooth muscle. In a later paper (1937c) they show that the denervated muscle of the nictitating membrane is also electrically excitable, but to a lesser extent than the normal musculature.

Bozler (1938a) shows that visceral smooth muscle can be stimulated directly by electric current and that the excitability of this type of muscle differs only quantitatively from nerve and striated muscle, as indicated by measurements of the strength, duration, and relationship of the rate of conduction and the refractory period. In a later paper (1938b) he states that "the propagation of an impulse in smooth muscle is accompanied by an action potential which, except for its slowness, is not different from that of nerve." Again (1939a) he demonstrates that all types of activity of

intestinal muscle are accompanied by bursts of brief action potentials which do not differ to any extent from those of voluntary muscle.

Luco and Rosenblueth (1939) describe experiments in which transmission of the motor nerve impulse may be fatigued in conditions in which neither the electrical excitability of muscle nor the spike-potentials of the nerve are impaired. They state that these results are incompatible with the electrical theory of neuromuscular transmission, but are in agreement with the chemical theory of mediation by acetylcholine.

Monnier (1936) outlines both the physical and the chemical theories of neuromuscular transmission, together with their respective limitations, and concludes that the two corresponding mechanisms are not opposed, but appear to exist simultaneously in the same neuromuscular system, one or the other predominating more or less, according to the type of muscle studied. Forbes (1936) adds that, "since the action potential of the nerve fiber is unable to excite adjacent fibers, we may suppose that it cannot excite adjacent cells, and that in this fact lies the need of a chemical mediator."

Parker (1940), in discussing the mode of innervation of chromatophores of the lower vertebrates, describes the dual innervation and the humoral control of these structures. He states that neurohormones, while they undoubtedly serve in synaptic transmission, probably do not act alone, but that the electrical transmission may also occur. However, he adds that the humoral control is predominant.

D. Neuromuscular transmission. Eccles and Magladery (1937b), in experiments conducted on the rhythmic responses of smooth muscle, conclude that rhythmic waves in the smooth muscle of the nictitating membrane are produced by the rhythmic discharge of all-or-nothing impulses in the units of the smooth

muscle. They add that the coördination of the rhythmic responses of the unit is not due to true syncytial connections of these smooth muscle cells, but probably to the tendency for a beat in one unit to set up beats in adjacent units.

E. Pharmacology. Henderson and Roepke (1937) review the drugs affecting the parasympathetic nerves. They find that atropine diminished or prevented the normal effect of nerve stimulation, and that hyoscyamine and hyoscine are more active than atropine. Homatropine is shown to increase cardiac rate and to prevent the effect of vagus stimulation, to dilate the pupil and to remove the effects of muscarine. Muscarine causes a decrease in cardiac rate, temporary constriction of the pupil, increased intestinal activity, dilation of the vessels of the rabbit's ear, and expulsion of urine and faeces. It has been shown to stimulate the inhibitory vagus endings. Pilocarpine causes either an arrest or a decrease in cardiac rate, and may inhibit vagus effects. The pupil contracts; the gut, uterus and urinary bladder show increased activity and spasm. Arecoline acts as does pilocarpine, but is more active than the latter. Physostigmine has numerous effects on the heart beat and on the action of the vagus on the heart; it contracts the pupil and the bronchial muscles, and causes contraction and increased peristalsis in the stomach and intestines.

Morison and Lissák (1938) explain the mode of action of piperidinomethylbenzodioxane (933F), stating that it destroys adrenalin in vitro, and thus blocks adrenergic impulses.

Lorman (1941) shows that thyroid acts on the vegetative nervous system to increase vasomotor activity, peristaltic activity and activity of the sweat glands.

Adriani and Rovenstine published, in 1941, an article on the autonomic responses of bronchial tissue to various anesthetic drugs. They find that cyclopropane, amytal, nembutal, evipal and pentothal have a constrictor effect on bronchial musculature, which could be prevented by previously adding atropine and relieved by the later additions of atropine. Eserine enhanced the constrictor effect. Ethylene, nitrous oxide and paraldehyde have either a mild constrictor action or no effect; this action is not modified by either eserine or atropine. Chloroform, ethyl ether and divinyl ether produce bronchial relaxation; the last anesthetic is reversed in its action by ergotamine.

F. Denervation. It is well known that denervation sensitizes the response of smooth muscle to stimuli. Luco (1937) shows this in inhibited structures (the response of intestine and uterus to adrenalin); Eccles and Magladery (1937c), on smooth muscle in general (reaction to direct stimulation); and Youmans (1938) for adrenalin and inhibitory sympathin on the intestine. Eccles and Magladery (1937c) also show that the sensitization of denervated muscle to adrenalin is decreased, and Simeone (1938) has found this to be true for the cat's nictitating membrane.

Cannon (1939) proposes the following law of denervation: "When in a series of efferent neurones a unit is destroyed, an increased irritability to chemical agents develops in the isolated structure or structures, the effect being maximal in the part directly denervated."

Klopp (1940) describes the reactions of partially denervated smooth muscle, with results which indicate that "degeneration of some of the postganglionic fibers which supply the nictitating membrane causes a relatively sharp division in the muscle. Some cells retain their normal sensitivity to sympathin and adrenalin while others are more sensitive. Such differentiation is

absent in normal or totally decentralized or denervated membranes."

Finally, Simeone (1937) shows that with regeneration of the nerve supply the increased sensitivity begins to decline.

DISCUSSION

It is evident from this thesis on the relation between autonomic nerve and smooth muscle cell that no definite conclusions can be made concerning either the anatomy or the physiology of this relationship. In discussing the physiology of neuromuscular transmission, the two theories were outlined briefly. The chemical theory states that there are two types of autonomic nerves, adrenergic and cholinergic, and that impulses are carried from neuron to neuron or from neuron to effector by the mediation of either acetylcholine or an adrenalin-like substance, sympathin. The recognition of two sympathins, an inhibitory and an excitatory one, was also discussed. The electrical theory, on the other hand, postulates that the electrical changes produced during conduction in the nerve serve in the transmission of the impulse across the synapse. Some investigators believe that both mechanisms may be involved, and that when the nerve impulse fails to reach adjacent nerve or muscle cells, the chemical mediator may intervene and complete the transmission.

Also discussed were the action of various drugs on the autonomic neuroeffector system, and the experiments on denervation. The latter seem to show that after severing the nerve supply to smooth muscle the tissue becomes more sensitive to mechanical stimuli and less sensitive to chemical stimuli.

In the anatomy of the relation between smooth muscle and nerve, there is still a great deal of disagreement. This is undoubtedly due to the different methods used by many workers, both as to staining technique and even as to the animal used as the source of the tissue.

The earlier workers used a silver nitrate technique, either that of

Cajal, or Bielschowsky's modification. With this technique, the neurofibrils are demonstrated, and the stain often affects the nerve terminations so that these appear as complicated structures (variously described as rosary-like terminal endings, end-rings, end-knobs, end-nets, a delicate veil). Some authors claim that these are definite structures of the nerve; others say that they are artifacts caused by the staining procedure.

Other investigators have used a technique of staining intravitally with methylene blue, and Jordan (1937) says that "this technique is invaluable for the demonstration of the terminal nerve supply of tissues." Using this stain, definite terminal nerve structures are not frequently seen. More often the nerves lose their neurilemmar sheath as they approach the effector cells, and end on or actually in the cells, the neurofibrillae penetrating the cell membrane to end in relation to the nucleus. The presence of neurofibrillae in the cells has also been demonstrated with the Bielschowsky technique.

It is apparent that the true significance of nerve endings has not been determined as yet, for if it were established, interpretations of the preparations made with the two techniques would concur, and not disagree. The only structures which seem to have been found with both stains are the neurofibrillae within the muscle cells; therefore it is my opinion that explanations based on the presence of these elements must be valid.

It is evidently agreed that, in addition to any plexuses of extrinsic origin, or other extrinsic innervation, every region of the smooth musculature innervated by the autonomic nervous system contains a terminal reticulum formed by the interstitial cells of Cajal. Earlier authors have stated that these cells are connective tissue. However, the demonstration of Nissl

bodies (Leeuwe, 1937) and neurofibrillae (Lawrentjew, 1925, and others) would seem to indicate, but not prove, that these are true nervous elements.

One obvious discrepancy in the reports of the various authors is that involving the number of cells directly in contact with an autonomic nerve fiber. Many authors (Evans, 1926; Boeke, 1932; S. L. Clark, 1937; Li, 1940) report a one to one relationship between nerve fiber and smooth muscle cell; others (Irwin, 1931; Kleyntjens and Langworthy, 1937; Gruber, 1938; Klopp, 1940) report that each muscle fiber does not receive a nerve ending, but that only one in from twenty-five to one hundred is directly innervated. Tiegs (1924) reports two nerve fibers to each muscle cell; other authors do not confirm this statement. These reports are based on observations made on different tissues (ciliary muscle, ureter, urinary bladder, gut, intrinsic muscles of the eye) and it is possible that the richness of the innervation may differ in the various organs; it is also possible that the staining might not have been as complete in some preparations as in others.

The nature of the musculature itself is evidently not thoroughly understood, as the discussion of the syncytial nature of the smooth muscle indicates; evidently, there is room for more research in this field.

From a consideration of the muscular nature of the pericapillary cells, it is evident that a need exists for further study and for exact definition of the vessels studied. It seems probable to me that Zweifach's classification of the smaller vessels as arterio-venous bridges, which are muscular, and true capillaries, which have no contractile pericytes, is justifiable; and also that the report of sphincter mechanisms at the point of capillary origin, which is based on experiments on living tissue, is also reliable.

Thus it may be stated that there is a need for more research in the field

of the autonomic innervation of smooth muscle; for definition of the nature of the tissue studied; and for a common technique in the staining of preparations before any theories can be agreed upon.

SUMMARY

In this paper the recent work dealing with the autonomic innervation of smooth muscle has been reviewed. The anatomy of the autonomic nervous system was discussed briefly, and the nature of smooth muscle, both the individual cells and the tissues, was described. The nature of the pericapillary cells was also discussed, and a brief mention was made of the physiology of nerve-free smooth muscle.

Boeke (1940) has described three theories of sympathetic nervous end formation: first, the postganglionic fibers go directly and independently to innervate the effector cells; second, the interstitial cells of Cajal intervene between the postganglionic cells and the effectors; and third, a gradual transition occurs from a neurofibrillar network to a terminal reticulum, a delicate network found everywhere in the body. The evidence for these theories has been discussed, and the innervation of the smooth muscular tissue in all areas of the body, the vascular system, the urogenital system, the gastro-intestinal tract, the eyes and the respiratory system, has been described. In considering the evidence, it would appear that all three theories may be involved in the actual innervation of smooth muscle; that the postganglionic fibers are in contact with Cajal's interstitial cells, which are syncytial in nature and form a network which is present in all areas of the body.

The physiology of the autonomic nervous system, particularly the work of Cannon and Rosenblueth and their co-workers, has also been discussed briefly. They describe the dual innervation of many smooth muscle cells from neurons of both the sympathetic and the parasympathetic systems; the theories of chemical mediation of nerve transmission, which postulate that acetylcholine and two sympathins, sympathin E and sympathin I, are released in the transmission of impulses from neuron to neuron, or from neuron to muscle fiber;

the electrical phenomena in transmission; the stages in neuromuscular transmission, together with the actions of various drugs in blocking transmission; and the sensitization produced by interrupting the nervous supply to smooth muscle. The more recent work in these fields was also outlined briefly.

The variability in experimental methods among the workers was discussed, and the different opinions regarding the autonomic innervation of smooth muscle were attributed to these discrepancies.

ABSTRACT

A thesis reviewing the recent work concerning the autonomic innervation of smooth muscle cells has been prepared, and without the support of individual investigations by the author a few conclusions have been proposed.

The nature of the gross anatomy of the autonomic nervous system is well-known, and is not discussed in the paper. The function of the autonomic system is briefly described as the "maintenance of stability or homeostasis in the fluid matrix of the organism." (Cannon and Rosenblueth, 1937)

There are found in the literature two theories which deal with the nature of smooth muscle cells. The work of McGill (1907, 1909) and Tiegs (1924) was presented as evidence for the action of neurofibrillae in contraction. Observations of McGill (1907, 1909), Evans (1926), Bozler (1938, 1939) and others indicated that the smooth musculature of the viscera, and particularly that of the uterus, is syncytial, and that conduction from muscle cell to muscle cell takes place directly through interprotoplasmic bridges. Fulton and Lutz (1942) have described a vascular smooth muscle syncytium, discontinuous at the junctions of the vessels, in the retrolingual membrane of the frog. On the other hand, the work of Tiegs (1924), Eccles and Magladery (1937b) and Cannon and Rosenblueth (1937) showed that in certain areas of the smooth musculature, particularly in the nictitating membrane, there is no evidence for the presence of a syncytium. In this case the transfer of impulses from cell to cell is probably humoral. It may be concluded that there are two types of smooth muscle, one syncytial and the other consisting of independent cells, and that these occur in characteristic regions of the body.

The various mechanisms for the contraction of capillaries are also discussed in the literature. Clark and Clark (1925b, 1940), Florey and Carleton

(1926), Michels (1936) and Zweifach (1934, 1937) have found no evidence for the dependence of the capillaries on the Rouget cells (pericytes, pericapillary cells) for contraction; on the other hand, Vimtrup (1922), Bensley and Vimtrup (1928), Jones (1936), and Fulton and Lutz (1940, 1941, 1942) presented experiments on living tissue and observations on fixed preparations as evidence for the contractility of certain pericapillary cells (the Rouget cells). Zweifach (1937) suggested a classification of the capillaries based on the structure of their walls. Thus he described arterio-venous bridges, which are muscular and actively contractile, and true capillaries, which are endothelial, and do not possess muscular perivascular elements.

In connection with the distribution of smooth muscle cells in the body, the papers of Ingalls (1923), Macklin (1929) and Acheson (1938) were outlined. Ingalls described the embryology and the anatomy of the dilator and the sphincter muscles of the iris; Macklin classified the pulmonary musculature as bronchial and interstitial, and defined the limits of both; and Acheson stated that the musculature of the nictitating membrane does not extend to its outer limit but only to its origin.

The differences in the physiology of smooth muscle from that of striated muscle were discussed briefly, and the work of Ferguson (1940) on the nerve-free smooth muscle of the chick amnion was presented here.

There are three broad theories concerning the morphological relations between smooth muscle cell and nerve fiber, and the monograph by Boeke (1940) describes them briefly. The first theory states that the postganglionic fibers go directly and independently to innervate the effector cells. The second theory says that the interstitial cells of Cajal intervene between the postganglionic fiber and the effector; and the third theory describes a grad-

ual transition from a neuro-fibrillar network into a terminal reticulum which is apparently present everywhere in the body. The work of many authors in relation to these theories was presented, and it would appear that the real nature of the autonomic innervation of smooth muscle may involve a combination of these three theories. Thus it appears that the postganglionic fibers are in contact with Cajal's interstitial cells, which are syncytial in nature, and that the fibers of these cells are in contact with, and may form, a delicate terminal reticulum of nerve fibers throughout the body.

Regional descriptions of the innervation of the smooth muscle of the various systems of the body were given. In the vascular musculature, Larsell (1921, 1922), J. D. Boyd (1937, 1941) and others describe endings on the muscle cells of arteries and arterioles; Michels (1935, 1936) and Larsell and Dow (1933) were unable to find endings on either the endothelium or the pericytes of capillaries, though many have shown that these smaller vessels are accompanied by nerves. Boeke (1932) reported that in the vessels of the human eye the nerve fibers extended to the muscle cells and continued into an extremely delicate network within them.

In the urogenital musculature, the work of Gruber (1938), Stewart (1937), Kleyntjens and Langworthy (1937), Langworthy and Murphy (1939), Davis (1933) and others was outlined. No nerve endings were found in the ureter or the uterus, but were demonstrated in the bladder and the fallopian tube.

Nerve endings in the gut musculature were found by Carpenter (1918), Hill (1927), Irwin (1931) and Li (1940). The plexuses in the walls of the gastrointestinal tract were described by Langley (1921), Hill (1927), van Esveld (1928), Schabadasch (1930), Irwin (1931), McSwiney (1931) and Li (1940). These plexuses, while independent, are connected to the vagus and sympathetic

fibers. The majority of these authors also indicate that the interstitial cells of Cajal are to be considered as the end-point of the enteric system.

Plexuses around the sphincter and dilator muscles of the iris were described by Boeke (1933), Jones (1932) and S. L. Clark (1937), and endings on or in the cells of these muscles were also described. The work of Larsell (1921, 1922), Larsell and Mason (1921), Larsell and Dow (1933) and Macklin (1929) defined plexuses in the respiratory musculature; nerve terminations are also described.

Cannon and Rosenblueth (1937), in dealing with the physiology of the autonomic neuro-effector system, describe the dual innervation of the smooth musculature from neurons of both the sympathetic and parasympathetic systems, and repeat the well-known fact that this innervation is antagonistic. The theories of chemical mediation are discussed; these lead to the belief that autonomic impulses are transmitted from neuron to neuron and from neuron to plain muscle fiber by the intervention of either acetylcholine or an adrenalin-like substance, sympathin. Also, there are apparently two sympathins, an inhibitory one and an excitatory one. The significance of the relative stability of sympathin and the rapid destruction of acetylcholine in the functions of the sympathetic and the parasympathetic systems is discussed. Also mentioned is the theory of electrical transmission of impulses; this postulates that the electrical changes produced during conduction in the nerve serve in the transmission of the impulse across the synapse. It is probable that both mechanisms are involved, and that when the electrical impulse fails to reach adjacent nerve or muscle cells the chemical mediator may intervene to complete the transmission. The steps in the transmission of impulses from neuron to muscle fiber are outlined briefly, and the action of various drugs in

blocking this transmission is described. The experiments on denervation are also discussed; these indicate that the severing of the nerve supply to the effector increases its response to mechanical stimulation and may decrease its response to electrical and chemical stimulation.

In criticizing the discrepancies in the results on the morphology of autonomic innervation of smooth muscles, the variations in staining techniques, together with the results obtained with silver and with methylene blue techniques, is discussed. It is concluded that the work on this problem is by no means finished, and that consistent results can be produced most reliably with similar methods.

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